Intrinsic and extrinsic factors associated with range of motion (ROM) with an emphasis on a novel genetic factor

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Declaration

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Abbreviations

bp	base pair
BMI	Body Mass Index
cm	Centimeter
ER	External rotation
Flex.	Flexibility
Hz	Hertz
hr	Hour
hrs/wk	Hours per week
IR	Internal Rotation
kg	Kilogram
km	Kilometer
min	Minutes
min/wk	Minutes per week
ml	Milliliter
mm	Millimeter
RFLP	Restriction fragment length polymorphism
ROM	Range of motion
Sh	Shoulder
SLR	Straight leg raise
SNP	Single nucleotide polymorphism
SR	Sit and reach
TR	Total rotation

U.V.	Ultraviolet
V	Volts
vs	Versus
%	Percentage
°C	Degrees Celcius
>	Greater than
<	Less than
≥	Greater than or equal to

Abstract

Introduction: Although there are numerous health benefits associated with participating in regular physical activity, there is also an increased risk of sustaining injuries, in particular musculoskeletal soft tissue injuries. Both an increased and decreased joint range of motion (ROM) has been reported as one of the intrinsic risk factors for these injuries. Similarly to injury, the ROM trait has also been associated with various extrinsic and intrinsic factors. Extrinsic factors that are associated with ROM include level and type of sports participation and temperature. Intrinsic factors include age, gender, limb dominance, weight/BMI, height, prior injury, flexibility training, ethnicity and genotype. It has been reported that ROM is a largely (47-70%) heritable trait in both pathological and apparently healthy populations. Mutations within the *COL5A1* gene cause classic Ehlers-Danlos Syndrome (EDS) which present with, among other clinical signs, generalised joint hypermobility. Furthermore, a *COL5A1* gene sequence variant, the *Bst*UI Restriction Fragment Length Polymorphism (RFLP), has previously been shown to be associated with ROM measurements in a cohort containing individuals with a history of Achilles tendon injuries.

Objectives: The aim of this study was, therefore, to investigate the association between the *COL5A1 Bst*UI (C/T) and *Dpn*II (C/T) RFLPs, as well as non-genetic intrinsic and extrinsic factors, and ROM measurements in an apparently healthy and physically active population.

Methods: The sit and reach (SR), passive straight leg raise (SLR) and shoulder internal (ShIR) and external rotation (ShER) assessments were performed on 325 (204 males, 121 females) white, apparently healthy and physically active subjects. Subjects were genotyped for the *Bst*UI (SNP rs12722) and *Dpn*II (SNP rs13946) RFLPs within the 3'-untranslated region (UTR) of the *COL5A1* gene. Level and type of sport participation, age, gender, limb dominance, height, weight, BMI, waist circumference, prior injury and flexibility training were also recorded to investigate possible associations with ROM.

Results: There was a significant interaction between age and *COL5A1 Bst*UI genotype with SR ROM. Subjects with a CC genotype were "protected" against the commonly reported age-related decline in SR ROM. This divergence in response to aging resulted in a significant difference in the mean SR ROM between the *Bst*UI RFLP genotype groups of the "old" (≥35 years) (TT=225 ± 96 mm, TC=245 ± 100 mm, CC=321 ± 108 mm, N=96, p=0.017), but not the "young" (<35 years) (N=197, p=0.626) subjects. While the *Dpn*II RFLP displayed a similar pattern of divergence in SR ROM with aging, this interaction was not significant. Nevertheless, the SR means were significantly different between *Dpn*II genotypes in the "old" group when the TT and TC genotypes (T allele) were combined and compared against the CC genotype (T allele=244 ± 98 mm, CC genotype=332 ± 15 mm, N=93, p=0.032). Furthermore, flexibility training (stretching) was associated with increased ROM only in the *Bst*UI TT genotype, suggesting a genotype-specific response. Of all the intrinsic and extrinsic factors

investigated in this cohort, only gender and genotype (either *Bst*UI or *Dpn*II RFLPs) were shown to contribute to SR ROM variance through multivariate analysis. Some inconsistent associations with intrinsic and extrinsic factors were observed with the SLR and shoulder ROM assessments, although small sample size and poor reliability of these measures made the results difficult to interpret with confidence.

Conclusion: The significant interaction of *COL5A1 Bst*UI RFLP genotype with age explains the differences in SR ROM measurements observed in older, but not younger, apparently healthy and physically active individuals. A similar, non-significant pattern in the DpnII RFLP resulted in significantly different SR ROM for the T allele in comparison to the CC genotype. Besides genotype, gender also an contributed significantly to SR ROM variance in the “old” cohort. Genetic sequence variants, in conjunction with commonly listed non-genetic intrinsic and extrinsic factors, need to be considered in order to understand the observed variance in ROM in apparently healthy and physically active populations.

Keywords: *COL5A1* genotype, range of motion (ROM), apparently healthy and physically active population, intrinsic and extrinsic factors, age, flexibility training.

Chapter 1

Extrinsic and Intrinsic Factors associated with range of motion (ROM) - A review

1.1 Introduction

Regular participation in physical exercise offers numerous health benefits to the individual and as a result is becoming increasingly popular [2;3]. However, participation in physical activity is not without risk of injury to the individual. Injuries, as a result of participation in physical activity for health, as well as various recreational and competitive sports, are therefore commonly seen by clinicians in athletic [3;4], military [5] and general populations [6].

While injuries during physical activity can occur at any anatomical site, the musculoskeletal system is most often affected, particularly in adults [7]. With over 100 million musculoskeletal injuries being recorded worldwide annually [4], the requirement for good quality research in this area is evident.

As a result of the high incidence of sports injuries in general, injury prevention strategies to reduce this high incidence have been proposed (Figure 1.1) [3]. One of these strategies is referred to as the “sequence of prevention” and involves four defined steps: 1. establishment of the extent of the injury problem, 2.

establishing aetiology of *mechanism* of injury, 3. introduction of a preventative measure, and 4. assessing the effectiveness of the intervention by repeating step 1. Extensive research in understanding the aetiology and mechanisms of sports injuries (step 2 in figure 1.1) has resulted in the identification of a number of risk factors associated with these injuries. These factors have been divided into extrinsic (external or environmental) and intrinsic (internal or physiological) factors [8] (Figure 1.2).

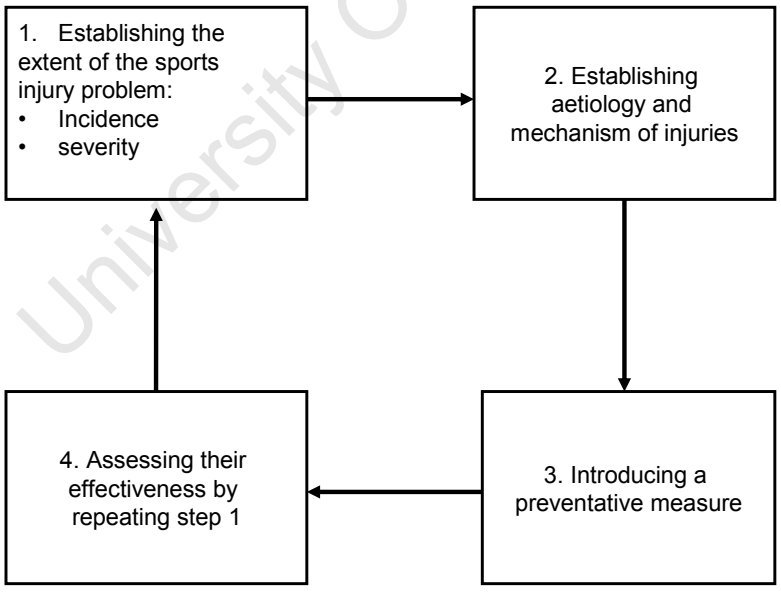

Figure 1.1: 'Sequence of prevention" model initially proposed by van Mechelen et al. [3].

As illustrated in Figure 1.2, the dynamic multifactorial model for the aetiology of sports injuries, which was originally proposed by Meeuwisse [9] and subsequently adapted by, amongst others, Bahr and Holme [8] demonstrates that an individual may be predisposed to a specific injury as a result of a combination of various intrinsic risk factors. Generally, these factors include age, gender, body composition (e.g. body weight, fat mass), health (e.g. history of previous injury, joint instability), physical fitness (e.g. muscle strength/power, maximal oxygen uptake, **joint range of motion**), anatomy (e.g. alignment of intercondylar notch width) and skill level (e.g. sport-specific technique, level of play). An individual may become predisposed to injury through a poorly understood combination of these *intrinsic risk factors*. Once **predisposed**, the individual can become a **susceptible** athlete through exposure to *extrinsic risk factors*. Broadly, these extrinsic risk factors include human factors (e.g. team mates, opponents, referees), protective equipment (e.g. sport's helmet, shin guards), sports equipment (e.g. skis) and environment (e.g. weather, type of playing surface). However, these two groups of risk factors alone may not necessarily result in an injury – an **inciting event** is a necessary extrinsic factor required to cause an injury, in particular an acute injury. An example of an inciting event could be any one of the following: joint motion (e.g. joint forces and moments), playing situation (e.g. skill performed), training program or match schedule. While the distinction into extrinsic and intrinsic factors makes for an easily interpretable model, a more practical classification for the sports clinician would be to divide risk factors into **modifiable** and **non-modifiable** risk factors. Bahr and Holme, in

their review of injury risk factors [8], state that strength, balance and range of motion ("flexibility") are important factors to investigate as they are examples of **modifiable** risks for injury (modifiable risk factors are highlighted in bold in figure 1.2). In contrast, factors such as age and prior injury are factors that are **non-modifiable** by nature.

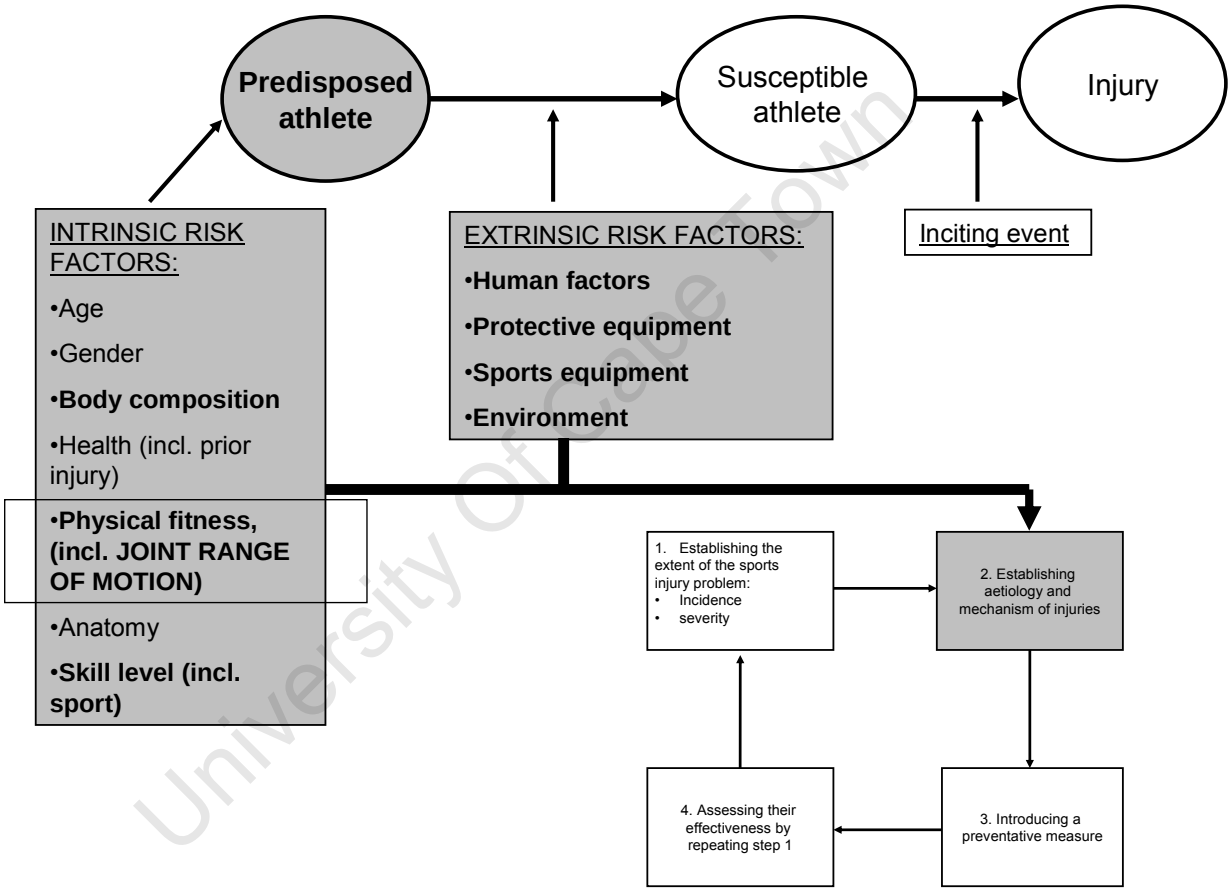

Figure 1.2. A modified model incorporating the "Dynamic model of sports injury aetiology [8]" into the four step injury prevention model [3]. Intrinsic and extrinsic factors that have been identified to predispose an athlete to injury are listed. Modifiable factors are emphasized with bold font.

Therefore, it is the **modifiable** risk factors that are of interest to the clinician as these present opportunities to prevent the high incidence of injury [10] (Step 3 of the "Sequence of prevention"). Once determined, these modifiable risk factors can then be investigated for associated factors various research methods. These studies are able to provide clinicians with a complete picture of potential injury mechanism. Range of motion (ROM) is modifiable in a wide range of population groups (Section 1.6.2.8) and has also frequently been linked to injury (Section 1.5) The focus of this dissertation is to identify extrinsic and intrinsic factors associated with ROM, in particular the genetic variants associated with this trait.

However, before investigating these factors, the commonly used terms in the literature in this area of research will first be defined and discussed in Section 1.2, followed by a description of the methods of measuring ROM (including a discussion on the validity and reliability of these methods) in Section 1.3. The continuum or distribution of range of motion will be discussed in Section 1.5. Before the factors associated with ROM are discussed (Section 1.6), this trait will first be investigated to assess whether it is a valid risk factor for musculoskeletal injury (Section 1.5). A final conclusion will be presented in Section 1.7.

1.2 Definition of terms

Flexibility is defined as the "range of motion (ROM) of a joint or series of joints that are influenced by muscles, tendons, ligaments, bones and bony structures" [11]. This is also known as "static flexibility" [12]. As a result of this definition, the terms flexibility and ROM are often used interchangeably.

ROM can be both static and dynamic [12]. Dynamic ROM refers to the "ease of movement within the obtainable ROM [12]". The "obtainable ROM" referred to in this definition is static ROM, which has been defined previously. "Joint laxity" or "joint hyperlaxity" is a function of the joint capsule and ligaments and does not involve the muscle. Practically, however, it may be difficult to remove or separate the influence of these structures and therefore this term should not be used synonymously with "hypermobility" (defined later) [12]. "Stiffness" is the resistance of a structure to deformation – in this case a passive stretch (defined in Section 1.3) [12]. Technically, this can only be measured through a dynamic ROM assessment (discussed in Section 1.3), although many authors use this term to describe reduced ROM. The term "tightness" is also often used to describe a deficit in ROM [13]. "Extensibility" is the ability of a musculotendinous unit to lengthen. "Elasticity" is the property of tissues that allows the tissue to return to its original length once a load has been removed. "Flexibility training" refers to regular participation in activities that aim to increase the ROM of a joint or group of joints [14].

Therefore, it is evident that many terms can, and have been used previously, to describe ROM, and the aspects of this trait. However, for the purposes of this dissertation, the term **range of motion (ROM)** will be used preferentially to describe the indirect measure of the extensibility of the tissues that have an influence over a particular joint [14]. ROM describes the functional movement of a specific joint or series of joints and is a measurable trait. “Hypermobility”, unless otherwise stated, will be used to describe an excess of ROM - in comparison to population-specific norms - in a particular joint (or series of joints). Conversely, “reduced ROM” will be used to describe a joint (or series of joints) with less joint ROM in comparison to population-specific norms.

1.3 Measuring ROM – instruments, reliability and validity

Two types of ROM can be measured – static or dynamic ROM. The measurement of static ROM provides an indirect measure of the extensibility of the tissues that have an influence over a particular joint [14]. The measurement of dynamic ROM is complex and requires a variety of assessment tools to measure features such as the passive torque generation or oscillation curves of the joint. There are relatively few studies that have measured this form of ROM and therefore this assessment will not be discussed further.

A variety of techniques are used to measure ROM. The assessments are graded according to the **reliability** and **validity** of the technique [15]. **Reliability** refers to the consistency of the technique and can be quantified through statistical analyses. A test-retest study design, conducted over a relatively short period of time is the most common method of assessment of a particular technique. ROM assessments are classified as either passive - researcher/clinician assisted assessment - or active – unassisted [15]. Passive/assisted assessments are reported to be more difficult to measure reliably than active/unassisted assessments [15]. The inter-tester and intra-tester reliability of the assessment should also be considered. Intra-tester reliability tends to be far greater than inter-tester reliability and thus the same researcher should conduct the assessments on all the subjects/patients in an investigation whenever possible [15]. The reliability can be reported through a variety of statistical methods, each with their own strengths and weaknesses. An intra-class correlation (ICC) and Pearson's correlation are two correlative analyses that are commonly used to assess reliability. A Bland-Altman 95% limits of agreement (LOA) analysis is "an indicator of absolute reliability" [16]. The LOA are calculated as two standard deviations either side of the mean of the difference between two tests on the subjects. Therefore, this reliability assessment tool is able to quantify the difference that a particular assessment tool is capable of detecting.

In contrast, **validity** provides an idea of how accurately as assessment actually measures what it purports to be measuring. For example, the straight leg raise

(SLR) test is reported, by some authors, to measure hamstring muscle length/hamstring muscle ROM. However, the only way to confirm this statement would be concurrent radiographic assessment of these measurements. The dynamic nature of ROM assessments limits such an investigation. Therefore, researchers must be satisfied with the fact that these ROM assessments provide an **indirect** measure of ROM at a particular joint. However, there will always be structures, besides the joint of interest that influences the assessment. The influence of these structures can only be minimized, although not eliminated altogether [15]. Therefore, validity is generally not assessed by the researchers of a particular study - only provided by authors as justification for using a particular method. On the other hand, the reliability of a particular ROM assessment needs to be assessed as this can vary depending on the tester and study population. Both the reliability and validity of the chosen ROM assessments should be provided by the authors of a good quality paper.

ROM assessment techniques that have been validated previously tend to be used more ubiquitously than others. The straight leg raise (SLR), sit and reach (SR) test and active knee extension (AKE) test are examples of three such assessments of the lower body. All three assessments have been validated for providing indirect assessments of hamstring ROM. However, the two most reliable - the SLR and SR [14;17] will be used in this dissertation and are therefore described in more detail in Chapter 2, Section 2.2. Similarly for the upper body, the internal and external rotation ROM assessments are examples of very

reliable ROM measures [18;19] and will be used in this dissertation (Chapter 2, Section 2.2). The data collected from less commonly used techniques do not provide results that are comparable with other ROM studies. For example, some researchers allocate an overall "flexibility" score to a subject/patient. This is done by summating the individual scores awarded to assessments of individual joints. Examples of an overall "flexibility" score are the "TIGHT" score [20], Flexitest [21] and Total Peripheral Score [22].

As previously indicated, the exact methodology and conditions during a musculoskeletal assessment, should be reported by the authors in good quality research. Testing conditions should be controlled and as similar as possible for each subject to maintain good reliability [15]. For example, researchers should control for a warm-up as joint ROM can be increased for up to three minutes after stretching [23]. Furthermore, the value obtained for an assessment changes with the number of times the assessment is performed. The largest changes occur over the first few assessments and then gradually level-off [24]. Thus, taking an average of the first few assessments could increase the reliability of the measurement [15]. Further, the temperature of the assessment environment, whether the subject has muscle stiffness from a recent sporting activity and how the subject/patient got to the testing venue are all important factors that could influence the assessment and should therefore be controlled for. However, not only acute factors influence ROM measures, regular stretching [25;26], participation

in certain sporting events [27;28], and chronic pain/injury [29] can also permanently influence ROM measures.

The **instruments** used for a particular assessment are also important to consider when examining the reliability and validity of the technique used. Three tools are recognized to measure flexibility accurately: a goniometer (goniometry), Leighton Flexometer ® (flexometry) and ELGON (electrogoniometry) [11]. A goniometer consists of a 180-degree protractor with extendable arms that can be locked in position to read both a starting and finishing range of motion. The Leighton Flexometer has a counterbalanced weight that points vertically at all times. Once the device is strapped on to the end of the limb of interest, a rotating dial provides a reading of the limb angle with respect to the perpendicular. The ELGON is a protractor that provides an electrical signal directly proportional to the angle of a particular joint [11]. However, the standardization of testing procedures appears to be more important than the instrument of choice [15].

The complexity of the particular joint being measured is also important to consider when comparing the reliability of the ROM measure. Certain joints, such as the wrist, are far less reliably assessed than simpler joints, such as the hip joint [15].

The use of the most clinically reliable and valid methods, in conjunction with the most accurate instruments, will produce the most accurate ROM data possible.

1.4 The continuum of ROM

Joint ROM ranges are assumed to follow a Gaussian distribution in an apparently healthy population [30] (Figure 1.3). Both reduced and increased ROM may present an increased risk for sustaining a muscular injury [31]. Heritable

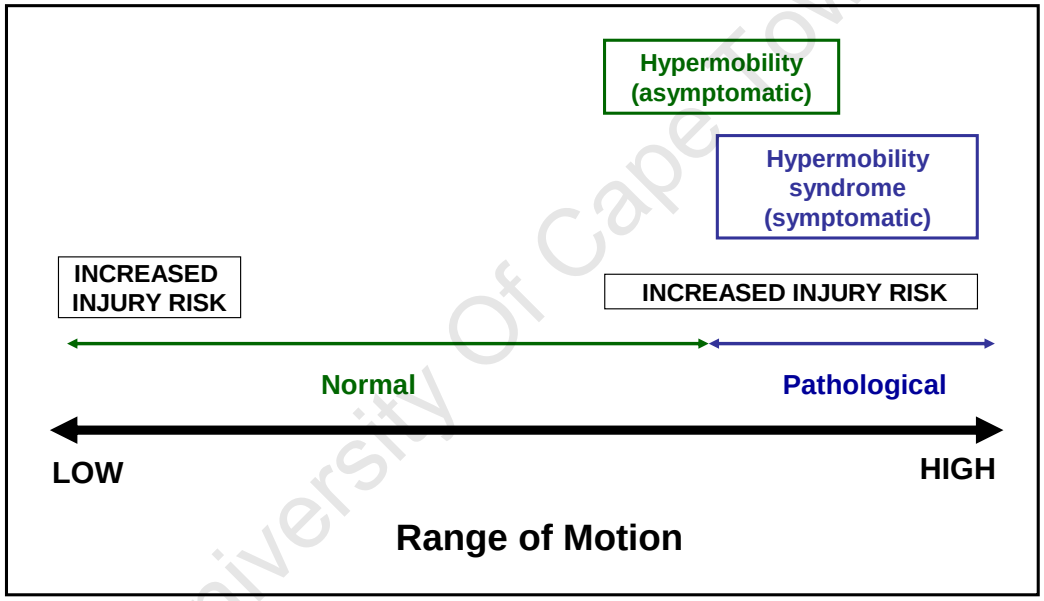

Figure 1.3. The range of motion continuum

disorders of connective tissue (HDCTs) comprise a group of pathologies that, while symptomatically diverse, present with a unifying symptom of generalised hypermobility [32]. Examples of HDCTs include Ehlers-Danlos Syndrome (EDS),

Marfan Syndrome (MS), Osteogenesis Imperfecta (OI) and Benign Joint Hypermobility Syndrome (BJHS) [30;32]. Not every individual confirmed to be hypermobile will necessarily have a hypermobility **syndrome**. Joint hypermobility needs to be symptomatic before the term "hypermobility syndrome" can accurately be decreed [32]. BJHS is so named as a result of its relatively mild symptoms in comparison to other more severe HDCTs.

Various diagnostic criteria are used to confirm hypermobility by clinicians. The most widely-used of these is the nine-point Beighton hypermobility score (Table 1.1) [1] which assesses the ability to perform five specific tasks. Each task is assigned a point based on whether the patient can perform the task or not (on both sides, except for final task), providing a total score out of nine. A score of four or more out of nine is confirmation of generalized hypermobility [1]. However, the fact that this method of identification requires a clinical examination means that not every hypermobile individual in the population is always identified. Thus, there is a chance that some individuals with hypermobile syndrome exist within the "normal" population (hence the overlap in Figure 1.3).

Table 1.1. Nine point Beighton hypermobility score assessment sheet [1].

The ability to:	Right	Left
1.Passively flex the fifth metacarpophalangeal joint to ≥ 90°	1	1
2. Oppose the thumb to the volar aspect of the ipsilateral forearm	1	1
3. Hyperextend the elbow to ≥ 10°	1	1
4. Hyperextend the knee to ≥ 10°	1	1
5. Place hands flat on floor without bending the knees	1	
TOTAL	**9**	

To reduce the chance of these hypermobile individuals remaining undiagnosed, two prominent rheumatology authors have recently validated a five-part questionnaire (Table 1.2) [33] that is sensitive and specific enough to determine hypermobility without the need of a clinical assessment. The answering of "yes" to two or more questions is confirmation of hypermobility.

Table 1.2. Five-part questionnaire to assess Benign Joint Hypermobility Syndrome (BJHS) [1].

1. Can you now (or could you ever) place your hands flat on the floor without bending your knees?
2. Can you now (or could you ever) bend your thumb to touch your forearm?
3. As a child did you amuse your friends by contorting your body into strange shapes OR could you do the splits?
4. As a child or teenager did your shoulder or kneecap dislocate on more than one occasion?
5. Do you consider yourself double-jointed?

It is estimated that asymptomatic hypermobility is present in 2-35% of males and between 5-57% of the females and varies according to age, gender and ethnicity. Hypermobility is more common in children than adults, in females than males, and Africans/Asians than Caucasians [1].

1.5 The association between ROM and risk of muscle injury, with specific reference to hamstring strains.

ROM has been implicated as a risk factor for general injuries, in a variety of populations. However, the results of these some of these reports are conflicting. Gleim [12] and Hoskins [34] concluded that a link between muscle "tightness" and injury may exist, but further research would be required to draw definite conclusions on this matter. Jones and Knapik [35] stated that both low **and** high levels of "flexibility" were associated with a high risk of injury in military populations. Taimela et al. [36] drew the same conclusion from an athletic civilian population. Worrell [37] concluded that a low level of "flexibility" was associated with a higher risk of hamstring injury in soccer players. The reason for this lack of unity in conclusions is due, in part, to the complex nature of injuries (Section 1.1), and in part to authors attempting to generalize a joint-specific trait. The chance that general ROM is related to a particular injury is unlikely as ROM is a complex phenotype, affected by numerous factors that make it a joint-specific trait (Section 1.6). An examination of ROM as a risk factor for all traditional sports-related injuries is beyond the scope of this dissertation. Therefore, for the

purposes of this thesis the author has focused on studies that examine hamstring ROM (at least as one of the ROM assessments) as a predictor of musculotendinous injury.

The justification for choosing hamstring muscle ROM to investigate as a risk factor for injury is two fold. Firstly, hamstring ROM is commonly assessed through reliable ROM techniques described in the previous section; secondly hamstring muscle strains are a very common musculoskeletal soft-tissue injury in a variety of sports-related activities and thus have been researched extensively [13;38-42]. Prospective cohorts offer the best methods to investigate potential risk factors for injury as thus a literature search was conducted examining ROM as a predictor of hamstring injury. The prospective studies found from a PUBMED literature search are summarized in Table 1.3. These studies were examined and rated by two criteria (Table 1.3), by the same author (James Brown – JB). Firstly, studies were allocated a **Level of Evidence** score according to Evidence Based Medicine criteria [43]. For a prospective study, a Level of Evidence of I is allocated if more than 80% of the original subjects were followed up. A study with follow-up of less than 80%, or if the follow-up numbers were not provided, was allocated a Level II. Furthermore, a '**magnitude of net benefit**' score [44] was subjectively assigned to each study based on the quality and description of the research (JB). Finally, an overall level of certainty for the association between hamstring flexibility and injury was allocated by examining **all** the papers ranked by these two criteria. Based on these methods of assessment, hamstring ROM carries a

high level of certainty as a risk factor for musculotendinous injury (Table 1.1). Furthermore, ten out of the twelve (83%) studies investigating hamstring ROM prospectively concluded that it was a significant predictor of injury.

Table 1.3. Summary of prospective cohort studies investigating hamstring ROM as a predictor of muscle strain or injury.

Authors	Level of evidence [43] and (certainty) [44]	Population studied	Joint/structure	Method of investigation	Definition of injury and medical personnel that provided the diagnosis*	Conclusion
Jones et al. [31] 1993	I (A)	Army recruits (male)	Hamstrings	SR (standing)	Lower extremity injuries (army physician - author)	Both ↓ and ↑ ROM an injury risk ($p \leq 0.05$)
Krivickas et al. [20] 1996	I (B)	College athletes (male and females)	Peripheral joints – "tightness" and "laxity".	"Tightness" - "TIGHT" score "Laxity" - Beighton Score	Back or lower extremity injury (trainer + team physician)	Both tightness ($p = 0.04$) and laxity ($p = 0.008$) associated with injury in MALES only.
Witvrouw et al. [45] 2003	II (B)	Professional soccer players (male)	ROM of hamstrings, quadriceps, adductor and gastrocnemius muscles	Passive SLR with goniometer.	Any tissue damage to lower extremities causing practice/game time loss (physical therapist - author)	Reduced hamstring ROM is a sig. ($P = 0.02$) predictor of injury in male soccer players.
Knapik et al. [46] 2001	I (A)	Army recruits in U.S (male and female)	Hamstring ROM	SR test – sitting with both legs extended simultaneously (standard), following warm-up.	Bodily damage resulting in medical assistance (health care provider). Heat and cold injuries as well as animal bites excluded.	High ($p = 0.05$) and low ($p=0.02$) levels ROM associated with injury in MALES only.
Lysens et al. [47] 1989	I (C)	Freshman physical education students	Muscle "tightness" and "joint laxity" all body joints.	Hamstrings- palm to floor/SR test (standing); "Laxity" – knee joint	Incident occurring during sports-workout resulting in ≥ 3 day absence from sport (sports medicine physician)	**Accident prone** males -high degree of ROM.; **overuse-prone** males – low degree of ROM, high "ligamentous laxity".
Diaz et al. [48] 1993	II (B)	Spanish Air Force soldiers (male)	Beighton criteria for joint laxity	Hamstring – palm to floor test/SR(standing)	Lesions/alterations to locomotor system (clinician)	"Lax" and "hyperlax" individuals more likely ($p < 0.5$) to develop injury than "normal".
Gabbe et al. [40] 2005	I (A)	Community level Australian Rules Football (male?)	Hamstring, quadriceps and iliopsoas ROM; lumbar spine, dorsiflexion and hip ROM; neural mobility	Hamstring – AKE, passive SLR and SR test (standard)	Hamstring injury – acute pain in onset in thigh, tenderness on palpation, pain on stretching or contraction of muscle (club physio/medical staff)	Hamstring ROM - AKE ($p = 0.076$), not passive SLR or SR test is a predictor of hamstring injury
Henderson et al. [49] 2009	I (A)	Elite soccer players (male)	Hip flexion/hamstring flexibility	Active and passive SLR following standardized warm-up	Hamstring injury – result in player not being able to train for ≥ 48 hours (club physio, doctor and sports therapist)	Active ROM was a significant predictor of injury. Both active and passive SLR were higher in non-injured (not significantly).
Yeung et al. [50] 2009	II (D)	Amateur and college sprinters (up to 400m)	Hamstring flexibility	Passive SLR following 10 min warm-up	Hamstring injury resulting in forced abstinence from training for ≥ 24 hours (physiotherapist – authors).	Flexibility not significantly different between injured/non-injured groups.
Gabbe et al. [51] 2006	I (D)	Elite Australian Rule Football (male?)	Lower extremity ROM and muscle flexibility; neural mobility	Hamstring – AKE and SR test (standard)	AFL injury database – hamstring injury resulting in ≥ 1 missed game (team doctor)	Hamstring flexibility not associated with hamstring injury.
Bradley and Portas [251}, 2007	I (A)	Elite (EPL) soccer players (male)	Hip Extensors and flexors, knee extensors and flexors, ankle dorsiflexors and	Hamstring – knee extension and flexion.	Musculotendinous damage to lower extremity sustained during training/competition that prevented	Injured players has sig. ($p<0.05$) less preseason knee and hip ROM than non-injured.

			plantar flexors.		normal participation in training/competition. (team medical staff/state-registered physio)	
Ekstrand and Gillquist	I (A)	Senior soccer division (male)	Hip Flexion, extension and abduction. Knee flexion and ankle dorsiflexion (hip abduction measured with goniometer, others measured with flexometer).	Hamstrings – hip flexion and knee flexion	Injury that occurred during match/practice and caused player to miss match/practice (orthopaedic surgeon).	Only players with reduced hip abduction ROM more likely to develop injury.

BJSM – British Journal of Sports Medicine; SJSMM – Scandinavian Journal of Medicine and Science in Sport; AJSM – American Journal of Sports Medicine

SR - sit and reach; SLR – Straight Leg Raise;

Only prospective studies investigating hamstring ROM as well as "hypermobility" as a risk factor for injury were included in this table. For the purposes of this table the crude measure of lumbar/hamstring "flexibility", the sit and reach test, is included as a measure of hamstring ROM.

Subjective scoring based on description of methods (including instruments used, warm-up or not, etc), reliability/repeatability of methods including whether this information was displayed, comparability of methods used.

*the authors were involved in diagnosing the injury

Level of Evidence Key [43]: I - High quality prospective study (all patients were enrolled at same point in their disease with ≥80% follow-up of enrolled patients) II - Lesser quality prospective study (e.g. patients enrolled at different points in their disease of <80% follow-up).

Magnitude of net benefit key [44]: A - substantial, B - Moderate, C - Small, D - negative/zero.

Therefore, the finding that ROM are a risk of injury justifies the investigation of ROM as a trait in general. While numerous theories have been presented to explain the mechanism of muscle injury as a result of altered ROM, the full investigation of these is also beyond the scope of this dissertation. In brief, two forms of hamstring strain have been proposed by Worrell [37] - (1) an acute onset, with associated pain, and (2) slow, insidious onset with preceding muscle "tightness" often reported before the actual injury. Strains are reported to occur most frequently after a particularly fatiguing training or match [37]. Since the primary aim of this dissertation is to identify extrinsic and intrinsic factors associated with ROM, the remainder of this review will focus on the factors that ultimately contribute to an individual's ROM

1.6 Factors associated with joint ROM

Much like the risk of injury, ROM in a joint or group of joints is associated with various intrinsic and extrinsic factors. Unlike the risk of injury, studies investigating these factors are all only observational/cross-sectional in nature. As a result, while the same criteria were applied to evaluate these studies as for Table 1.1 (Level of Evidence[43] and "magnitude of net benefit"[44]), the subjective evaluation of these studies focuses more on the reporting of methods and the choice of ROM assessments (refer to Section 1.3) than the type of study. Extrinsic factors associated with joint ROM include level and type of activity performed and environmental conditions, such as temperature. Intrinsic factors

include age, gender, weight, muscle size, limb dominance, prior injury, flexibility training, (although this may possess both extrinsic **and** intrinsic properties), ethnicity and, more recently, genotype.

1.6.1 Extrinsic factors associated with ROM.

A summary and analysis of the studies that have reported an association of the level and type of activity performed, as well as, temperature, is summarized in table 1 of Appendix A.

1.6.1.1 Level and type of activity performed

It was suggested in three reviews that the level and type of activity are two factors that may influence an individual's ROM [11;12;28]. Numerous cross-sectional studies [13;27;29;52-55] have confirmed that the **type** of activity influences joint-specific ROM [12]. In fact, significant ROM differences have been discovered even when comparing the different positions **within** certain sports such as American Football [56] or soccer [57]. The control for the investigated joint of interest in these investigations was either a non-athletic population or the contralateral arm of the subject. Overhead activities, such as tennis and baseball, are associated with a decrease in internal rotation ROM of the glenohumeral joint [28;55;58]. Furthermore, the throwing action of baseball is associated with increased external ROM in comparison to a non-throwing arm [28]. Experienced long-distance runners [27] and

elite soccer players [13;54], have been associated with reduced ROM in lower extremity measures. Cyclists [57] and sprinters [59] have also been associated with reduced lower extremity ROM in comparison to a normal population [60], although these two studies did not have a control group for comparison.

In contrast, sports that perform flexibility training for performance exhibit enhanced ROM. Aikido athletes have increased ROM in comparison to both upper- and lower-body athletes as a result of their particular training [29]. Dancers [52] and gymnasts [53] are two groups of athletes that are consistently associated with enhanced ROM. It can be concluded from these cross-sectional studies that, based on the requirements of that particular activity, different sports are associated with ROM alterations at specific joints. These alterations in ROM can occur through regular flexibility training, as in dancing and the performing arts [52], or through repeated loading activities (such as throwing) required by the particular sport [55;58]. However, this explanation is still open to debate as these cross-sectional studies are not able to infer a cause-effect relationship.

A potential confounding factor in this particular area of research is the significant overrepresentation of joint hypermobility in the performing arts. In fact, hypermobility has actually been suggested as a positive selection factor in this sporting sphere [1]. Whether this overrepresentation of hypermobility is a result of sport-specific training or whether it is inherited, and thus a positive selection factor from early on, is still open to debate. The identification of genetic variants

associated with ROM within these performing sports may eventually help to answer this question.

There are less cross-sectional studies available that investigate **level of sport participation**. However, one study suggested that reduced ROM conferred an advantage in running economy [61], although this is not the only factor that determines the level of an endurance athlete.

In conclusion, although there is a paucity of data describing the effect of the **level of sport participation** on ROM, the **type of sport** and even the position within certain sports has been associated with altered ROM measures. To generalize this association one could conclude that weight-bearing activities that require repeated loading (such as running and soccer) are associated with reduced lower extremity ROM with a HIGH level of certainty.

1.6.1.2 Environmental temperature

There is anecdotal evidence that environmental temperature has an influence on ROM measurements [11], but this has not been clearly demonstrated. A single study in humans showed that the direct application of a moist heat pack, which effectively raised hamstring muscle temperature by 0.4°C, did not have any effect on ROM in male subjects [62]. The only study to show an effect of temperature on

ROM was in rat muscle [63]. In this study, passive tension decreased with an increase in temperature from 10 to 35°C, implying an increase in ROM.

Thus, despite the assumption that environmental temperature affects ROM, this is yet to be shown. Therefore, increased ROM is associated with higher temperatures with a LOW level certainty.

1.6.2 Intrinsic factors associated with ROM

It has been suggested that several intrinsic factors are associated with joint ROM. These include age, gender, weight, muscle size, limb dominance, prior injury, flexibility training (although this may possess both extrinsic **and** intrinsic properties), ethnicity and genotype. A summary and analysis of studies that have investigated these intrinsic factors in presented in table A.2 of appendix A.

1.6.2.1 Age

In general, older populations have less ROM at various joints than their younger counterparts [64]. A review by Kell et al. [65] estimated the decline in joint ROM to be 20-30% between 30-70 years of age. A number of researchers have drawn the same conclusion in the general population [21;66-68], as well as various athletic

[10;53;55] populations. This age-dependent decline has been attributed to loss of tendon "flexibility" as a result of biochemical changes that occur to soft tissue structures with aging [69]. Others [64] have suggested that this decline is related to an expected reduction in physical activity with age. Of interest is that internal rotation of the shoulder joint tends to increase with age [66;70]. However, this is the only joint shown to be positively correlated with age.

In conclusion, increased age is associated with a general reduction in joint ROM with a HIGH level of certainty.

1.6.2.2 Gender

It is commonly reported that females tend to have greater joint ROM than their male counterparts [11;71], and are also more likely to be "hypermobile" [1]. Female and male joint ROM patterns appear to diverge during or just after puberty [71], but some authors have reported a difference in ROM measures between males and females from as early as 5 years of age [21]. These gender differences have been shown by various assessment techniques and has been reported in both general [21;66-68;72] and athletic populations [27;53;73]. In most studies, gender differences in ROM have been reported in the lower limb (e.g. hamstrings/hip ROM [27;67;68;73]), but gender differences have also been reported in the upper limbs [53;66;73]. In an endurance running population, Wang et al. [27] showed that both males and females had significantly reduced hamstring ROM in comparison to a non-

running population, but the female runners still had greater ROM than their male running counterparts.

In conclusion, the female gender is associated with increased joint ROM with a HIGH level of certainty.

1.6.2.3 Limb Dominance

Bearing in mind the effect of level and type of sport participation in Section 1.6.1.1, it would be logical to assume that training asymmetrically – i.e. the dominant limb more than the non-dominant – would result in noticeable differences between in joint ROM between these limbs. Evidence of this difference has been reported in upper-body sports such as baseball [74], tennis [53;73] and waterpolo [28] as well as lower-body sports, such as soccer [49]. However, this difference in ROM measures between limbs is also present in sports that train symmetrically– such as endurance running [27]. Of interest is the fact that this difference has been described in a number of reports in the general, non-athletic population in both the upper and lower body [27;66;75-77]. Barnes et al. [66] and Conte et al. [77] reported similar findings when examining shoulders in two separate, apparently healthy, non-athletic populations. In both populations, the dominant shoulder has significantly more external rotation, yet significantly less internal rotation compared to the non-dominant shoulder. It has been hypothesized, particularly in athletic populations that the observed difference between limbs

was attributable to repeated micro-trauma occurring to the dominant limb. However, the significant difference in ROM between dominant and non-dominant limbs described by Barnes et al. [66] in the youngest sample of their study population (0-10 years), suggests another mechanism underlies the divergence in limb ROM. Conte et al. [77] suggested that the difference is simply due to tissue adaptations as a result of differences in the frequency of use of both sides.

In a non-athletic population, Macedo and Magee [75] reported that 34 of 60 ROM measures were different when comparing dominant/non-dominant limbs. The differences ranged from 0.26° to 7.54°, leading the authors to conclude that these differences were not **clinically** significant for any measure. The fact that a degree of measurement error (dependent on the particular technique, assessor, joint, etc.) exists around most ROM measures supports these author's conclusion. This is an important finding as it enables clinicians to gain an idea of baseline flexibility following an injury to one side of the body. However, these ROM differences between limbs in **athletic** populations can be meaningfully different. When comparing runners to non-runners, Wang et al. [27] found a larger difference between dominant and non-dominant hamstring ROM in the running group. The difference was attributed to the increased demand of the muscle of the dominant leg. Furthermore, Knapik et al. [78] found a pre-season difference in ROM of greater than 15% between right and left legs to be correlated with a higher chance of sustaining an injury in female collegiate athletes. The authors

also concluded that a larger difference in ROM between limbs was associated with a greater risk of injury during the season.

Regardless of the reason for the observed difference, it is clear that hand/foot dominance is associated with altered joint ROM. External rotation of the dominant shoulder and internal rotation of the non-dominant shoulder are associated with increased ROM in comparison to the contralateral limb with a HIGH level of certainty. The dominant leg is associated with a reduced ROM in comparison to the non-dominant leg also with a HIGH level of certainty. While the demands of daily living in a non-athletic population may or may not result in clinically different ROM measures between limbs depending on the literature consulted, this difference **can be** clinically significant in an athletic population. The clinical significance is determined by the fact that this ROM difference between limbs can pose an injury risk to the athlete [78].

1.6.2.4 Prior Injury

The term “prior injury” is vague. The time delay between the injury occurrence and the ROM assessment might well affect the findings, although the term does not specify a particular time frame. Chronic injuries are not considered for this section as they constitute a “current injury”. While it is logical to assume that a soft tissue injury would result in a reduced ROM around a particular joint, this has not been consistently demonstrated in the literature. In most studies investigating

prior injury, particularly those reporting the injury in athletic populations, ROM was only assessed retrospectively and at the end of a competitive season. Sprinters who had suffered an injury during the season [59] displayed significantly less hamstring ROM than their uninjured counterparts when they were examined at the end of the season. However, previously injured elite soccer players [49], also examined at the end of a competitive season, did not exhibit reduced ROM. Furthermore, previously injured US fire-fighters [72] were not significantly less "mobile" than their uninjured counterparts.

While the nature of acute injuries makes them difficult to investigate, a case study [79] was produced by chance from a professional skier. The skier suffered an acute hamstring muscle strain while performing a maximal treadmill trial. Kinematics analyses that were being performed during this trial revealed only a very slight (1-2°) decrease in hip and knee ROM immediately after the incident. Furthermore, a five-year follow up study [80] examined loss of hip ROM at 2, 10, 21 and 42 days following a hamstring injury in sprinters and dancers. In this study, subjects were recruited retrospectively (i.e. post-injury), therefore the only means of comparison was the patient's contralateral limb. The main findings of this study were that ROM had returned to 90% of the contralateral limb's ROM after 42 days of injury, but that the time required to return to pre-injury performance levels was far longer. However, using the contralateral limb, particularly for athletic populations, is not always a good reference point as there can be clinically significant differences between dominant and non-dominant limbs (Section 1.6.2.3).

In conclusion, there is inconsistency in the relationship between prior injury and alterations in joint ROM. The main reasons for the inconsistency appear to be related to the vague nature of term “prior injury”. Furthermore, the nature of acute injuries means that good quality studies in this area are difficult to perform. Acute injuries are also difficult to assess prospectively as they will always lack statistical power as a result of their ‘by chance’ nature. When investigated retrospectively, these studies lack a true reference point for the limb pre-injury. While the loss in ROM resulting from acute injuries is not always significant the time delay before assessment may affect overall results. This decrease in ROM appears to be most severe in the few days after injury, but may still be present up to 7 weeks later. Therefore, in general, acute injuries are associated with a decreased ROM (possibly in reference to the contralateral limb) with a MODERATE level of certainty.

1.6.2.5 Weight/Body Mass Index (BMI)

The association between body weight or body mass index and joint ROM has been reported in a general population between the ages of 20 and 69 years [81]. In this study, baseline ROM measurements of 606 males and females were assessed using a standard SR test. The main findings of this study were that BMI, measured at baseline, was significantly negatively correlated with the SR test scores taken at the same time. A similar conclusion was drawn from a

general Japanese population [82] that was divided into three groups based on BMI, in which the SR test was also used as a proxy of hip ROM. In contrast, another study [83] found no relationship between BMI and hip ROM. However, the SR test was not used in this study, possibly explaining the discrepancy.

In summary, increased body weight and BMI are associated with reduced ROM (as assessed by the SR test). While only a few good quality studies have investigated these two factors, the consistency of results warrants the conclusion that increased body weight/BMI is associated with reduced ROM with a MODERATE level of certainty.

1.6.2.6 Height

The association between height and joint ROM has only been reported in one study. In this study of young athletes [84], increased height was significantly negatively associated with both knee extension and flexion test. While this is was a well designed investigation, it is the only study describing such an association. Thus, without more evidence, increased height is associated with a decreased ROM with a LOW level of certainty.

1.6.2.7 Muscle size

It has been suggested that increased muscle mass/size may be associated with reduced joint ROM [27]. However, there are a limited number of studies that have investigated this association. In one study, the muscle cross-sectional area of an elite-level orienteer was determined by 2D echo on Magnetic Resonance Images [85]. The main findings of this study were that increased lateral cross-sectional area of hamstrings was negatively correlated with ROM measures (toe-touch results), indicating that a larger hamstring muscle size may be associated with a reduced ROM.

In conclusion, the certainty that increased muscle size is associated with reduced ROM is LOW due to a lack of more supporting studies.

1.6.2.8 Flexibility training

Regular flexibility training is sometimes referred to as an extrinsic factor but will be considered as an intrinsic factor for the purposes of this dissertation. The justification for this decision revolves on the belief of the author that the **response** to flexibility training is dependent on the individual. It has been documented that flexibility training enhances ROM, irrespective of the population investigated or the particular method used [86;87]. Healthy populations, irrespective of age are all able to significantly increase ROM following a regular stretching

intervention [25;88-91]. Successful stretching protocols are diverse with regards to frequency, duration and form. In a recently published review, the regularity, rather than the exact protocol, of stretching appears to be most important for increasing ROM [87]. Individuals with "tight" hamstrings (according to Active Knee Extension test) were also responsive to flexibility training [88;89]. Despite dancers performing regular flexibility training, this cohort would not be ideal to study due to the potentially confounding prevalence of hypermobility in this sport (Section 1.6.2.7: *Level and type of activity performed*)

In conclusion, regular flexibility training is associated with increased joint ROM with a HIGH level of certainty.

1.6.2.9 Ethnicity

In one review [1] it has been suggested that asymptomatic "hypermobility" is more common in Asian and Black ethnic groups compared with Caucasians. However, to the author's knowledge, no cross-sectional studies have examined ethnic differences in joint ROM measurements. Therefore, ethnicity as a factor associated with joint ROM requires, further investigation.

1.6.2.10 Genotype

ROM is, at least in part, a heritable trait [92;93] – in fact its heritability has been estimated to be between 64% [92] and 70% [93] in classical twin studies. Heritable Disorders of Connective Tissue (HDCT) provide evidence of this heritability in a pathological population. HDCT is a broad classification of genetic disorders that have a unifying symptom of, amongst other clinical features, joint hypermobility. An extreme ("striking") phenotype, such as joint hypermobility, is useful to geneticists in locating regions of the genome that are closely linked to a particular phenotype through disease causing mutations [94]. For example, over half the patients who present with classic Ehlers-Danlos Syndrome (EDS), a common HDCT, possess a disease-causing mutation in the *COL5A1* gene [30].

This gene encodes for the alpha 1 chain of all the type V collagen isoforms. Type V collagen forms heterotypic fibres with type I collagen – the most prominent protein in connective tissue – where it is believed to play an important role in regulating fibre diameter [95]. While musculoskeletal soft tissues are comprised of a wide variety of proteins and other macromolecules (e.g. elastin, glycoproteins, proteoglycans and glycosaminoglycans), type I collagen and the other collagen types, such as type V collagen, are the most abundant structural proteins [30]. Furthermore, it has been suggested that sequence variants within the *COL5A1* gene, which encodes for the alpha 1 chain of type V collagen, may be associated with ROM in apparently healthy individuals [32]. This would appear a logical conclusion bearing in mind that ROM of a joint, or series of joints, is

influenced by tissues and structures, such as tendons and muscles, of that particular joint (Section 1.2).

To date only two publications have investigated the association of variants within genes and the normal variation in range of motion. Posthumus et al. [96] have shown that a functional variant within the *metallo-matrix proteinase 3* (*MMP3*) gene was not associated with lower limb ROM measurements. Collins et al. [97], on the other hand showed a significant association between the *Bst*UI Restriction Fragment Length Polymorphism (RFLP) within the 3'-untranslated region (UTR) of the *COL5A1* gene and both sit and reach and straight leg raise measurements. However, the authors [97] noted some limitations of this study – mainly that the majority of subjects had a previous history of Achilles tendon injury.

Owing to paucity of research and the divergent findings of the only papers in this area, the certainty that increased or decreased ROM is associated with certain genotypes is LOW.

1.7 Conclusion

Soft tissue injuries, particularly muscle strains, occur commonly while participating in sports and recreational activities [38;98]. Research in this area has been driven by the economic cost and loss of functional capacity as a result of

these avoidable traumas [3;8]. Underlying causes or factors associated with injury have been divided into extrinsic and intrinsic by nature [3] (Figure 1.2). ROM is an example of a modifiable intrinsic risk factor that is associated with injury. In the case of hamstring ROM, muscle injuries are associated with either increased or decreased ROM with a high level of certainty (refer to table 1.3).

There is a host of commonly-listed intrinsic and extrinsic factors associated with ROM. In this chapter, a literature search was performed to investigate the certainty with which all of these factors are associated with ROM, in order that they might get ranked. A summary of the findings of this classification process is presented in Table 1.4.

Table 1.4. Intrinsic and extrinsic factors and their certainty of association with ROM.

Intrinsic/extrinsic	Factor	Certainty
Extrinsic	Level and type of activity	High
	Temperature	Low
Intrinsic	Age	High
	Gender	High
	Limb dominance	High
	Flexibility training	High
	Prior injury	Moderate
	Weight/BMI	Moderate
	Height	Low
	Muscle size	Low
	Ethnicity	Insufficient evidence
	Genotype	Low

A complex interaction between these factors ultimately results in an individual's ROM phenotype. The factors that are associated with flexibility with a higher level of certainty are more likely to contribute to the final trait.

However, the confidence of this statement is only as strong as the research investigating the individual factors. A low level of certainty indicates that either the research in the area has produced inconsistent findings, has been performed poorly or that there is minimal published research in this area. Thus, the five

factors (temperature, height, muscle size, ethnicity and genotype) that were given the ranking of a LOW level of certainty of association (or have insufficient evidence for a certainty to be assigned) are ideal candidates for further research.

In conclusion, several intrinsic and extrinsic factors have been shown to be associated with joint ROM in a variety of populations. The focus of Chapter 2 will be to investigate many of these commonly-listed factors simultaneously for associations with ROM measures within an apparently healthy and physically active cohort.

Chapter 2

Investigation of common intrinsic and extrinsic factors associated with range of motion (ROM) in an apparently healthy active population

2.1 Introduction

As reviewed in the previous chapter (Chapter 1), an increased or decreased range of motion (ROM) is a common intrinsic risk factor for several musculoskeletal soft tissue injuries [99]. In addition, ROM is a complex phenotype that is also associated with both intrinsic and extrinsic factors. Commonly listed extrinsic factors are level and type of sport participation and temperature (Section 1.6.1). Commonly listed intrinsic factors associated with ROM (as opposed to injury) include age, gender, height, weight, BMI, waist circumference, muscle size, flexibility training, prior injury, limb dominance (Section 1.6.2). Less common intrinsic factors associated with ROM are ethnicity and genotype (Section 1.6.2). The possible ability of these intrinsic and extrinsic factors to affect general or joint-specific ROM of an individual through their interaction is poorly understood.

Of the extrinsic factors, temperature is generally positively correlated with ROM [11]. The inferred effect of sport participation (through cross-sectional studies) on ROM is very much sport-specific. As reviewed in section 1.6.1.1, participation in

some sports is associated with an increase in general and/or joint specific ROM (e.g. ballet) while participation in others is associated with a decrease in ROM, (e.g. road running). Some sports, such as baseball, are associated with both joint-specific hypermobility (external rotation of shoulder) and reduced ROM [28].

Of the intrinsic factors of age, weight/BMI, height and muscle size have generally been shown to be negatively correlated with ROM (section 1.6.2). Muscle size will not be investigated in this study for an association with ROM. Additionally, females have more ROM than males at all stages of life [65]. Furthermore, the presence of a prior injury is generally associated with reduced ROM [37]. In terms of comparing sides of the body, the lower body dominant limb is generally associated with reduced ROM in comparison to the non-dominant limb, although whether this difference is clinically meaningful is debatable [75]. Furthermore, external rotation of the dominant shoulder and internal rotation of the non-dominant shoulder are generally associated with more ROM than their contralateral limbs for the same respective measurements in both non-athletic [75;100] and athletic populations [28]. Waist circumference has not been well investigated as yet, and this factor will be investigated in the present chapter. Ethnicity and genotype have not been researched sufficiently and will be discussed further in Chapter 3.

Despite numerous cross-sectional studies investigating one or two of these factors at a time, no single study - to the author's knowledge - has attempted to

investigate multiple intrinsic and extrinsic factors simultaneously for associations with ROM in a well described cohort. Therefore, the purpose of the study presented in this chapter is to investigate whether these common intrinsic and extrinsic factors, when examined concurrently, are associated with upper and lower body ROM measures in a large, apparently healthy, uninjured and physically active cohort.

2.2 Methods

2.2.1 Type of study

This research study was a cross-sectional study.

2.2.2. Subjects

Three hundred and twenty-five apparently healthy and physically active males (N=204) and females (N=121) were recruited for this study. Subjects were recruited from 1) fitness centres/clubs and the student population of the University of Cape Town (N=197), and 2) two large road running events within the greater Cape Town region – the 56km Two Oceans Ultra-marathon (N=109) and the 42.2km Mr Price Winelands Marathon (N=19).

Inclusion criteria for subjects in this study were as follows: 1) being physically active (at least one hour per week), 2) being apparently healthy at the time of the study, 3) being non-obese (BMI ≥ 30 $kg.m^{-2}$ and waist circumference ≥88cm for females, ≥102cm for males), 4) being older than 18 years of age, and 5) being free from serious injury in the 24 months prior to testing. In athletic populations, injury is usually classified according to "time loss" of training or match play [101]. This definition can also be used in the workplace; however for ease of recall we adopted a different definition. "Serious injury" was defined for the purposes of this dissertation as an incident occurring to musculoskeletal tissue that required either hospitalization or immobilization. As ethnicity has been suggested to be associated with ROM, only white individuals were recruited for this study. This original study protocol was approved initially by the Human Research Ethics Committee of the Faculty of Health Sciences in 2008 (ref number 092/2008) within the University of Cape Town, South Africa (Appendix B1). An amendment to this original protocol was also approved in 2009 by the aforementioned committee, with the same reference number (Appendix B2).

2.2.3 Pre-Testing procedure

The testing protocol is summarized in figures 2.1 and 2.2. The details of these procedures are included in the text - sections 2.2.3 - section 2.2.5.

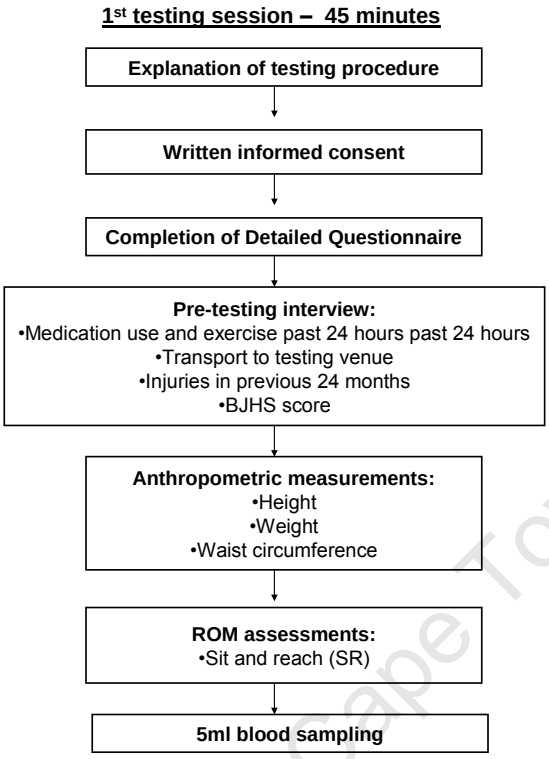

Figure 2.1. Detailed diagram of the order of testing of the first testing session. SR – sit and reach; BJHS – benign joint hypermobility syndrome; ROM – range of motion; ml – millilitres.

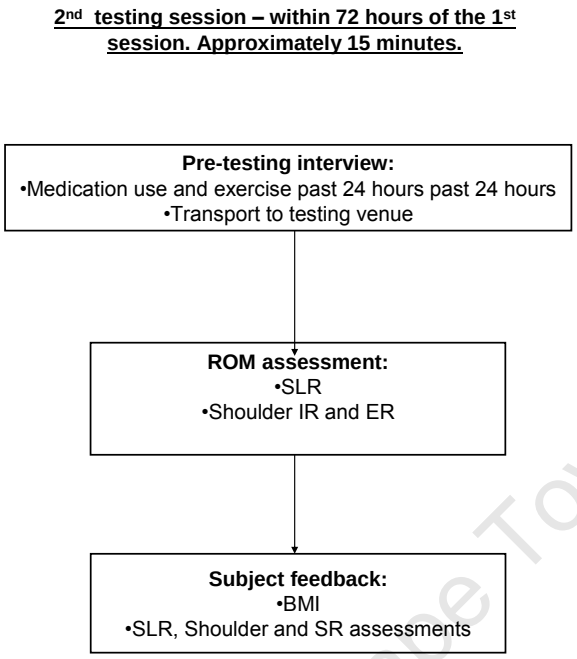

Figure 2.2. Detailed diagram indicating order of second testing session. ROM – range of motion; BMI – body mass index; SLR – straight leg raise; IR – internal rotation; ER – external rotation

Subjects were requested not to partake in any unaccustomed physical activity, stretch or use any medication in the 24 hours prior to testing, which has been shown to affect ROM, particularly in physical active individuals [102]. Once at the venue, the testing procedure was explained to the subject in depth. Prior to testing, subjects completed a written informed consent form (Appendix C). Subjects were also asked to complete a detailed questionnaire including

information on personal details, sports participation, personal medical/injury history and family medical/injury history (Appendix D1 and D2). The ROM assessments were to be performed without any form of a warm-up (i.e. “cold”). Therefore, subjects were asked a number of questions in a pre-testing interview to ascertain if their ROM may have been influenced in the 24 hours prior to testing. The following information was recorded on a data sheet (Appendix E) from the pre-testing interview: 1) how the subject arrived at the testing venue - to determine if a warming-up effect may have occurred from walking or cycling to the venue; 2) medication use in the past 24 hours – to ascertain whether the pain response to the stretching of ligaments/tendons had potentially been altered; 3) exercise/stretching activity in the past 24 hours – and, if so, the main limbs involved in the activity and whether it was accustomed or unaccustomed for that particular subject; 4) any current injuries (past two years); and 5) leg and arm dominance. Furthermore, the subjects were also asked the benign joint hypermobility syndrome (BJHS) five-part questionnaire (Table 1.2, Section 1.4). This questionnaire has been validated to identify hypermobile individuals with an 84% success rate [33]. The testing conditions, such as temperature and time of day were also recorded on the data sheet (Appendix E).

2.2.4 Anthropometric measurements

Weight, height and waist circumference were recorded by the same researcher (Caron-Jayne Miller - CM) during the first visit. Weight and height were measured simultaneously with a mounted stadiometer (County Scales Limited, Nottingham, United Kingdom). Weight measurements were taken with the subject wearing minimal clothing, such as a light t-shirt and a pair of shorts. Waist circumference was measured at the narrowest section of the torso, between the umbilicus and xiphoid process, with the subject standing in a relaxed position, arms at their sides and feet together [17]. This measure was recorded due to our obesity definition including a waist circumference stipulation.

2.2.5 Range of motion (ROM) assessments

Three clinically validated ROM assessments were performed on the subjects at two separate testing sessions at the Sports Science Institute of South Africa (N=197). Two lower body assessments, the sit and reach test [17] (SR) and straight leg raise test (SLR) [103], were performed at separate visits to prevent one influencing the other. Although subjects were requested to return for their second testing session at least 24 hours, but no more than ten days after the initial session, this was not always feasible (mean = 15 ± 22 days, range = 0 -95 days). However, the majority (64%) of subjects were tested for the second session

within 10 days of the first. Any changes in the non-serious injury status of the subject were noted at the second testing session pre-testing interview. The third ROM assessment, an upper body assessment, was the shoulder internal (IR) and external rotation (ER) in 90° abduction [18]. This assessment was always performed on the same day as the SLR. For the laboratory-based SR assessment (visit 1), the testing temperature ranged from 18 to 24°C (mean = 20.9 ± 1.3°C, N=275). Similarly, for the laboratory-based SLR/shoulder assessment (visit 2) the temperature ranged from 19 to 26°C (mean = 21.7 ± 1.6°C, N=55). Only the SR ROM measurement was performed on marathon runners (N=138). The temperature ranged from 26 to 32°C for the Mr Price Winelands Marathon study (mean = 29.9 ± 1.9°C, N=12), but was controlled for the Two Oceans cohort at 21°C (N=82).

The better of two attempts was accepted as the final score for the SR test [17]. The other assessments were performed only once to eliminate the effect of repeated measurements [24].

2.2.5.1 Sit and reach test

The sit and reach test is well documented as a measure of both hamstring musculotendinous unit length (indirect measure of hip ROM) and of lumbar ROM [17;104]. The sit and reach box was placed up against a solid object, such as a wall

or pillar. The subjects were asked to remove their shoes and then place their feet flat up against the footrest of the box. A ruler was mounted on the top of the box, with the 26cm mark of the ruler aligned parallel with the front of the footrest [17]. The 0cm mark of the ruler was closest to the subject, while the far end of the ruler (44cm) was closest to the solid foundation against which the box was braced. The subjects were instructed to reach as far forward as possible on the device, while maintaining full extension of their legs and with their feet against the footrest at all times. For a successful attempt the position on the ruler had to be held for two seconds and the subjects were not permitted to "bounce" forward. A two second break was permitted between consecutive attempts. The better of two attempts was recorded as the final value [17].

2.2.5.2 Straight Leg Raise (SLR)

The SLR assessment provides an indirect measure of hamstring ROM. It is advantageous as an assessment as it is both easy to perform and clinically acceptable [14]. Each subject was placed in a supine position on a plinth. Two CAM Walker® II (AliMed®, Massachusetts, USA) boots were then placed on the subject's feet to maintain the foot in a neutral position throughout the SLR test. A telemetric EMG system (Telemyo 900®, Noraxon, Arizona, USA) was used to determine the end range of motion (ROM) due to the high repeatability of this particular method [14]. Two disposable electrodes (Blue Sensor™ Medicotest,

Denmark) were placed on the belly of the Biceps Femoris muscle of each leg. Prior to placing the electrodes on the skin, the skin over the muscle was shaved and cleaned with ethanol. The placement and location of the electrodes were according to the recommendations by SENIAM (Surface EMG for Non-invasive Assessment of Muscles) [105]. Therefore, two electrodes were carefully taped to the belly of each muscle, parallel to the muscle fibres with an inter-electrode distance of 20mm. A telemetric signal was relayed to an antenna connected to an online computer and captured at 2000Hz. Before recording the EMG, each subject was asked to contract their muscles to verify the absence of crosstalk in the EMG signal. Furthermore, the machine was 'earthed' at the bony prominence of the knee to prevent excessive artefact interference with the recording.

A Leighton Flexometer™ (Leighton Flexometer Inc., Spokane, USA) was used to assess the change in ROM for this test. The instrument was attached to the test leg using an adjustable strap. Full knee extension was ensured throughout the procedure by the tester (JB) using one hand to provide gentle downward pressure onto the subject's knee throughout the procedure. To begin the test, one hand was placed under the heel of the test leg (CM), while the other hand operated the Flexometer™. The other tester (JB) maintained full extension of the test leg by applying light pressure onto the top of the patella, while the other hand ensured the non-test leg remained on the plinth to prevent hip rotation. The test leg was lifted at 30° per second, which was ensured by the one tester (JB) reading aloud the stopwatch on the EMG program. This rate was practiced on

three subjects prior to official testing. The end point of the SLR test was defined as the point at which a “spike” in EMG activity was observed by the tester (JB). This indicates the start of contraction of the hamstring, indicating the end-point of that ROM. If no spike was observed, then the tester (CM) terminated at end-feel, which is the next most reliable assessment of end ROM (r=O.95) [14]. The leg was then returned to its starting position at the same rate as it had been raised.

2.2.5.3 Shoulder Internal (IR) and external rotations (ER)

Shoulder IR and ER are the most reliable and valid methods in terms of inter- and intra-tester reliability [19], for measuring ROM associated with the shoulder joint. For both rotations, each subject was supine on a plinth with the shoulder in 90° abduction and the elbow in 90° flexion. The elbow was maintained in alignment with the shoulder throughout the procedure by gentle guidance from the tester. In the starting position (0°), the forearm was perpendicular to the ground with the subject’s fingers pointing towards the ceiling. The scapula was manually fixed by the tester (CM), and the arm rotated passively until end range. For external rotation, the arm was rotated towards the subject’s head, while for internal rotation the arm was rotated towards the subject’s stomach. Both assessments began in the neutral position (arm perpendicular to floor) and end range was determined by a cease in motion or a perception of movement in the scapula by the tester (CM). To determine the ROM for each of these

assessments, a Leighton Flexometer™ was attached to the subject's wrist. The angle at the start of the procedure was subtracted from the angle at the end of the procedure to determine final ROM for each assessment.

2.2.7 Statistical Analysis

Data collected from this study was analysed using STATISTICA (versions 8.0 and 9.0 - StatSoft Inc., Tulsa, OK, USA). Data that were not normally distributed (age, SR scores, Shoulder rotation scores, waist circumference, Two Oceans finishing time) were log transformed before analysis. These data were still not normally distributed and therefore both parametric and non-parametric analyses were run at all times. Furthermore, if a parametric test was performed on data that was not normally distributed, a Levene's Test of variation was used to determine homogeneity of the continuous data. An analysis of variance (ANOVA) test was used to determine whether significant differences exist between the means of continuous data (age, height, weight, flexibility training, ROM measurements) and gender or BJHS groups. Where appropriate, the p value of the ROM measurements was adjusted for gender, waist circumference, prior injury and exercise in the 24 hours prior to testing. This was due to significant influence of these factors on ROM measures (gender, waist circumference and exercise in past 24 hours) and age categories (prior injury). A chi-squared analysis was used to determine the frequency distribution of the two different

categorical data (e.g. gender and BJHS category) and a Fisher's exact test applied if one group had particularly low representations.

GraphPad Prism Version 5.02 was used for drawing and analysis of Pearson's correlation graphs. Linear regression lines were applied to determine the r-value (slope of the line) and deviation from zero of correlated data.

2.2.8 Repeatability of ROM assessments

Two repeatability studies were performed over the two year period (2008-2009), during which data were collected for this study to assess the consistency of the ROM assessments. The first was performed in May/June of 2008 (N=16) and the second in February/March of 2009 (N=9). The data of these tests were combined for analysis. One subject, in each repeatability assessment, was excluded from analyses for the following reasons: 1) In 2008 the subject was excluded due to having taken part in unaccustomed exercise in the 24 hours before the first, but not the second, testing session, 2) In 2009, the subject was excluded due to having cycled to the testing venue on the second, but not the first, testing session.

In general, the lower body assessments were far more repeatable than the upper body assessments in both repeatability studies (Table 2.1).

Table 2.1. Combined test-retest repeatability studies of 2008 and 2009 on a subset using correlations, calculated Intra-Class Correlations (ICCs) and calculated Bland-Altman limits of agreement. For all paired analyses, N=23 except for IR. dom. shoulder (N=22).

	Correlations		Bland-Altman test		
	ICC	**r**	**Mean - 2 S.D**	**Mean + 2 S.D**	**LOA**
SR	0.95	0.90 [a]	-65 mm	59 mm	124mm
Dom. SLR	0.95	0.92 [a]	-19°	17°	36°
Non-dom. SLR	0.93	0.86 [a]	-22°	18°	41°
Dom. ShIR [c]	0.70	0.56 [b]	-33°	47°	80°
Dom. ShER.	0.60	0.43 [b]	-22°	31°	53°
Non-dom. ShIR	0.59	0.44 [b]	-24°	33°	57°
Non-dom. ShER	0.45	0.38	-26°	43°	69°

[a] $p<0.05$
[b] $p<0.001$
[c] N=22
SR – sit and reach; SLR- straight leg raise; Dom – Dominant; Sh – shoulder; IR – internal Rotation, ER – external Rotation.
LOA - limits of agreement; S.D - standard deviation

The intraclass correlations (ICC) of the SR and dominant (dom.) and non-dominant (non-dom.) SLR assessments were comparably high with a range from 0.93-0.95. The shoulder ROM assessments, in general, had lower ICCs, with a range from 0.45 to 0.70. Correlative analyses revealed that the first and second tests were significantly correlated ($P<0.001$) for all the lower body assessments, and all the shoulder assessments, except for the non-dom. ShER. A Bland-Altman analysis provides an idea of absolute reliability (discussed in Chapter 1, Section 1.3). By comparing the difference against the mean score of between two

tests two consecutive tests, a range of limits of agreement (LOA) were revealed for the ROM techniques. For the lower body assessments, the SR had LOA of 124mm, while the SLR techniques had LOA of 36° and 41° for the dominant and non-dominant legs, respectively. Upper body assessments had differences in LOA that ranged from 53° to 80°. An example of a Bland-Altman analysis, for the two SR tests, is included in Appendix F, Figure F.1.

2.3 Results

2.3.1 General characteristics of subjects

The descriptive data of the study sample is examined in its entirety and separately by gender (Table 2.2). As expected, the mean age was not statistically different between males and females. However, all of the anthropometric variables (height, weight, BMI and waist circumference) were significantly larger in the males when compared to females ($p<0.001$). The range (minimum and maximum) in these anthropometric variables was relatively narrow: Height: 1.52 - 1.98m; Weight: 49.3 - 120.0 kg; BMI: 18.1 - 35.2kg/m^2; Waist circumference: 56 - 105cm. In contrast, age had a wider range (18 - 63 years).

Table 2.2. General characteristics of all the subjects, as well as a comparison between males and females

	All (N=325)	**Male** (N=204)	**Female** (N=121)	**p-value** [a]
Age (years)	32.0 ± 11.9 (321)	32.3 ± 11.6 (202)	31.4 ± 9.9 (119)	0.500
Height (m)	1.75 ± 0.09 (253)	1.80 ± 0.06 (157)	1.66 ± 0.06 (96)	**<0.001**
Weight (kg)	73.2 ± 13.2 (253)	79.7 ± 11.2 (157)	62.5 ± 8.2 (96)	**<0.001**
BMI (kg/m^2)	23.8 ± 2.9 (251)	24.5 ± 2.9 (155)	22.6 ± 2.5 (96)	**<0.001**
Waist circumference (cm)	78 ± 8.4 (157)	82.6 ± 7.6 (88)	72.1 ± 5.1 (69)	**<0.001**
Hand dominance (% right)	92.2 (191)	92.0 (112)	92.4 (79)	0.911
Foot dominance (% right)	93.7 (191)	92.9 (112)	95.0 (79)	0.560
Sitting (% of day)	57.0 ± 25.4 (235)	57.8 ± 24.8 (148)	55.5 ± 26.7 (87)	0.501
Students (%)	44.9 (219)	44.9 (136)	38.6 (83)	0.360
Current injury (%) [b]	25.1 (259)	26.7 (165)	22.3 (94)	0.440
CT injury/disorder (%)	47.1 (259)	52.7 (165)	37.2 (94)	**0.016**

Values are expressed as average ± standard deviation or as a frequency. The number of subjects (N) is in parentheses. Significant differences are in bold.
Age, height, weight, waist circumference and limb dominance were obtained or measured during the first visit. Body mass index (BMI) was calculated as kilograms per meter squared. Country of birth, occupation, limb dominance and injury data were self-reported in a questionnaire.
[a] male vs female.
[b] non-serious injuries that did not require hospitalization or immobilization.
m - meter; kg - kilogram; cm - centimetres; SA - South Africa; min - minutes; CT - connective tissue.

Of the 325 subjects, 79.7% (N=259) completed a version of the detailed personal details, family and medical history, flexibility training and sport history questionnaires (Appendix D1 and D2). On average, the subjects reported that

57.0% of each day was spent sitting and only 9.2% was spent performing manual labour. The subjects reported standing and walking for 21.4% and 19.8% of the day, respectively. There were no significant differences in the relative amount of time each day that males and females reported sitting (M: 57.8%, F: 55.5%, p=0.501), standing (M: 20.7%, F: 22.6%, p=0.491), or performing manual labour (M: 8.6%, F: 10.3%, p=0.437). Males (17.5%), however, reported spending significantly (p=0.041) less time of each day walking than females (21.5%).

The cohort consisted mainly of students (44.9%, N=219) and this finding was similar in the males (44.9%, N=136) and females (38.6%, N=83) (Table 2.1). In terms of self-reported daily activity, students were not significantly different to the rest of the sample. Students reported sitting for 55.0%, standing for 22.6%, walking for 18.8% and performing manual labour for 10.1% on average of each day. The rest of the sample (non-students) reported, on average, sitting for 56.8%, standing for 21.8%, walking 19.6% and performing manual labour for 9.2% of each day.

The majority of subjects reported being right hand (92.7%) and foot (94.0%) dominant. Those who reported being “ambidextrous” for either was asked to clarify which was there “stronger” hand or foot. By means of confirmation a version of this question was again asked in the self-reported detailed questionnaire. The right side was reported as being dominant for handedness in 90.8% (N=69) and for footedness in 86.3% (N=63) of the sample. For the

dominant leg, 2.7% (N=2) reported that they were ambidextrous in the self-reported questionnaire. There were no significant gender differences in the right hand (92.0% male, N=92; 92.4%, female, N=61; p=0.921), as well as right foot (93.0% male, N=93; 92.4% female, N=63; p=0.515) dominance (Table 2.1).

Approximately one quarter (25.1%, N=65) of the sample reported a current injury that did not require hospitalization or immobilization (Table 2.1). Of those reporting a non-serious injury, seven (2.7%) reported two current injuries. The frequency of injuries was similar in both genders (p=0.440). As expected, the average age of those reporting a current non-serious injury was significantly higher (34.5 ± 11.1 years, N=65) than those who did not report any injuries (30.0 ± 10.3 years, N=194; p=0.003). The incidence of current non-serious injuries were confirmed by the subjects during their first visit at the pre-testing interview (21.2%, N=60, p=0.416).

A detailed summary of all the reported injuries is presented in Tables F.1 and F.2 of Appendix F2. In summary, injuries were reported to have occurred to the right hand side 41.3% (N=26) of the time, while left hand side was reported in 42.9% (N=27) of reported injury cases (N=63). The majority of reported injuries were to the lower body (91.4%, N=64). The most commonly injured structure was a muscle (36.8%, N=28), followed by a ligament (22.4%, N=17), a tendon (18.4%, N=14), bone (10.5%, N=8) and a joint (5.5%, N=4) (Table 2.1). The most commonly reported anatomical site of injury was the knee (25.6%, N=20),

followed by the lower back (10.3%, N=8), “Achilles” (9.0%, N=7), hamstring or thigh (9.0%, N=7), shoulder (6.5%, N=5), hip (6.4%, N=5) and calf (6.4%, N=5). Males reported a statistically higher prevalence of a history of tendon or ligament injuries or connective tissue disorders (“CT injury/disorder”) than females (p=0.016) (Table 2.1). This injury would have to have occurred more than 24 months prior to testing for the subject to be included in the study. As a result of this definition, this injury data will not be considered as “prior injury” (a common intrinsic factor associated with ROM) for the purposes of this thesis.

Furthermore, the pre-testing interview ascertained various factors that may have influenced the ROM measures in the 24 hours prior to testing (Table 2.3).

Table 2.3: Factors potentially affected ROM in 24 hours before testing, assessed at pre-testing interview.

	1st testing session	2nd testing session
Transport		
Motorized transport (%)	94.0 (284)	100 (56)
Walk (%)	4.9 (284)	0 (56)
Cycle (%)	1.1 (284)	0 (56)
Exercise – past 24 hr		
Yes (%)	36.3 (292)	15.5 (57)
Lower Body (%) [a]	57.6 (85)	80.0 (10)
Typical (%) [a]	95.3 (85)	100 (10)
Stretch – past 24 hr		
Yes (%)	20.2 (168)	10.9 (55)
Lower Body (%) [a]	64.3 (28)	83.3 (8)
Medication use – past 24 hr		
Yes (%)	11.2 (277)	3.6 (55)
Pain Killers (%) [a]	45.4 (31)	100 (2)
Anti-inflammatories (%) [a]	9.1 (31)	0 (2)
Oral steroids (%) [a]	45.4 (31)	0 (2)

Values are expressed as average ± standard deviation or as a frequency. The number of subjects (N) is in parentheses.
SR - sit and reach; SLR - straight leg raise; Hr - hour
[a] Only a subset of the sample were asked these questions.
No statistical analyses were performed for 1st visit vs 2nd visit due to low sample numbers of 2nd visit.

The majority of subjects reported having travelled by motorized (car, motorcycle or public transport) transport to both testing sessions (1st session = 94%, N=267, 2nd session = 100%, N=56). Although a substantial proportion of subjects reported performing exercise in the 24 hours before being tested, the majority of this exercise was classified by subjects as "accustomed" or "typical" exercise (1st session = 95%, N=81, 2nd session = 100%, N=10). Furthermore, the majority of this exercise was reported as "Lower body" activity for both sessions (1st session = 58%, N=33, 2nd session = 80%, N=8). Fewer subjects reported stretching than did exercising in the 24 hours prior to testing. The majority of this stretching was reported to be to the lower body (1st session = 64.3%, N=18, 2nd session = 83%, N=7). Minimal subjects reported using medication in the 24 hours prior to testing. "Pain-killers" and oral steroids were the medications reported most frequently.

Importantly, the SR ROM was significantly less in those that reported exercising in the 24 hours before being tested (mean = 246 ± 112 mm, N=106) in comparison to those that did not report any exercise (mean = 274 ± 106 mm, N=186) (p=0.035). However, neither of the other two ROM assessments were significantly different for those reporting exercise or not in the 24 hours prior to testing. Similarly, there were no other significant differences for any of the three ROM measures when compared for any of these other factors recorded in Table 2.3 (data not shown).

2.3.2 Sport and flexibility training details of entire sample, as well as a comparison between males and females.

Of those who were recruited outside of running events, 96.4% (N=162) reported participation in at least one sport (Table 2.4). Of this group, 91.7% (N=154) reported participating in two, 68.5% (N=115) three, 38.7% (N=65) four, 26.2% (N=44) five, and 10.7% (N=18) six or more sports. The questionnaire for those that were recruited at running events did not permit the recording of sporting activities outside of running (Appendix D1). While a detailed analysis of all the reported sports was beyond the scope of this dissertation, this finding confirmed the cohort to be a physically active one, with an interest in a variety of sports. However, when analyzing the primary sport of the subjects, the majority (50.6%, N=82) reported that their primary sport was either (1) 19.1% running, (2) 14.8% rugby, (3) 10.5% hockey or (4) 6.2% cycling (Table 2.4). There were no significant differences between genders for the participation in either running ($p=0.084$) or cycling ($p=0.610$). However, no women reported participating in rugby ($p<0.001$). In contrast, females reported participating in hockey significantly more than males ($p=0.041$).

The subjects' primary sports were divided into categories based on whether it was associated with a decreased or increased lower body ROM. This categorization was based on peer-reviewed literature as well as a subjective interpretation of sports with similar demands on the lower body. If no literature

was available for a reported sport, or if the sport was not easily comparable with another referenced sport, the sport was categorized as “unknown”. Examples of sports that have been associated with a reduced lower body ROM are running [27], cycling [2] and soccer [13]. Examples of sports that have been associated with an increased ROM are dancing [52], and gymnastics [53]. Examples of unknown sports are lifesaving and horse-riding. It is acknowledged that the referenced studies are cross-sectional and therefore a cause-effect relationship cannot be inferred. However, these cross-sectional studies are able to generalise about the ROM of the athletes participating in that particular activity. A complete list of reported sports and their effect on ROM is included in Table F.3 in Appendix F. Owing to the high prevalence of participation in lower body-dominant sports (such as running, hockey and cycling), the association with lower rather than upper body ROM was focused on. Based on the available literature, the majority of subjects’ reported primary sports was associated with reduced ROM (75.9%, N=179) (Table 2.4). As summarised in table 2.4, the participation of males and females in these three different categories of sport was not statistically different.

On average this sample only reported performing 1.5 minutes of flexibility training per week (Table 2.4). However, only 47.6% (N=114) of the sample reported performing flexibility training. Males and females were not statistically different in the amount of flexibility training that they reported performing each week (p=0.853).

Table 2.4. Sport and flexibility training details of all subjects as well as a comparison between males and females.

	All	Male	Female	p-value [a]
Primary Sport [b]:				
Running (%)	19.1 (31)	14.3 (14)	26.6 (17)	0.084
Rugby (%)	14.8 (24)	24.5 (24)	0.0 (0)	**<0.001**
Hockey (%)	10.5 (17)	6.1 (6)	17.2 (11)	**0.041**
Cycling (%)	6.2 (10)	6.1 (6)	6.2 (4)	0.610
Sport participation, categorized by associated lower body ROM [c]:				
Increased ROM (%)	7.2 (17)	6.6 (10)	8.24 (7)	0.426
Decreased ROM (%)	75.9 (179)	79.5 (120)	69.4 (59)	0.294
Unknown (%)	17.0 (40)	13.9 (21)	22.4 (19)	0.114
Flexibility training [d] (min/week)	1.5 ± 3.7 (239)	1.6 ± 4.3 (153)	1.5 ± 2.3 (86)	0.853

Except for flexibility training which is expressed as an average ± standard deviation, all other values expressed as frequencies. The number of subjects (N) is in parentheses. Significant differences are in bold.

[a] Male vs Female

[b] Thirty-eight different sports were reported by the subjects as their "Primary Sport". The four sports listed in this table were reported far more frequently than the other 34 and hence were analysed separately.

[c] All reported sports were allocated into one of three categories based on their influence on lower body range of motion: increasing, decreasing or unknown effect. This division was based on available literature where possible, but is also largely subjective. The lower body was chosen instead of the upper body due to better repeatability of the former assessments technique (see Appendix F, Table F3).

[d] Calculated from four reported data: (1.) days per week, (2.) times per day, (3.) repetitions of each stretch and (4.) time that each stretch is held. Only 47.6% (N=246) of the population reported performing regular flexibility training – either before, during or after sport or at any other time.

ROM - range of motion; min - minutes

2.3.3 Prevalence of hypermobility and range of motion (ROM) measurements of the entire sample, as well as a comparison between males and females.

Almost one-third (33.1%, N=83) of the subjects were assessed to be clinically hypermobile by the BJHS five-part questionnaire [33] (Section 1.4, Table 1.2) (Table 2.5).

Table 2.5. Range of motion (ROM) measurements and hypermobility prevalence in the entire sample, including a comparison between males and females.

	All	Male	Female	*p*-value [a]
Hypermobile (%)	33.1 (83)	24.8 (38)	45.9 (45)	**0.001**
SR (mm)	264 ± 108 (315)	239 ± 101 (199)	306 ± 107 (116)	**<0.001**
Dom. SLR – (°)	89 ± 23 (61)	77 ± 16 (34)	104 ± 21 (27)	**<0.001**
Non-dom. SLR – (°)	84 ± 23 (61)	73 ± 19 (34)	97 ± 21 (27)	**<0.001**
Dom. Shoulder IR – (°)	93 ± 17 (177)	89± 16 (103)	98 ± 17 (74)	**0.001**
Dom. Shoulder ER – (°)	105 ± 16 (179)	101 ± 15 (103)	112 ± 15 (76)	**<0.001**
Dom. Shoulder TR – (°) [b]	198 ± 27 (178)	190 ± 24 (103)	208 ± 29 (75)	**<0.001**
Non-dom. Shoulder IR – (°)	105 ± 17 (179)	103 ± 16 (103)	107 ± 18 (76)	0.180
Non-dom. Shoulder ER – (°)	99 ± 18 (179)	93 ± 15 (103)	107 ± 18 (76)	**<0.001**
Non-dom. Shoulder TR – (°) [b]	204 ± 29 (179)	197 ± 26 (103)	214 ± 30 (76)	**<0.001**

With the exception of hypermobile, which is expressed as a frequency, all other values are expressed as averages ± standard deviations. The number of subjects (N) is in parentheses. Significant differences are in bold.
[a] Males vs Females
[b] Total rotation (TR) of the shoulder was calculated as the sum of the internal rotation (IR) and external rotation (ER).
Dom. - dominant; SR - sit and reach; SLR - straight leg raise.
Hypermobility was defined as subjects answering positively to two or more of the five questions in the BJHS five-part questionnaire. Females were significantly more likely to be assessed as clinically hypermobile than males (p=0.001).

With the exception of the IR of the non-dominant shoulder, all ROM measures were significantly greater in females than males ($p<0.001$) (Table 2.5). As a result of this difference in ROM in males and females, data were analysed separately or covaried for gender throughout the remainder of this chapter.

Dominant and non-dominant limbs were significantly different for both upper and lower body ROM assessments (Table 2.6). The dominant leg (89 ± 22°) had significantly ($p=0.012$) more ROM than the non-dominant (84 ± 23°) leg, as assessed by the SLR. Similarly, dom. shoulder ER (105 ± 16°) had significantly ($p<0.001$) more ROM than the non-dominant shoulder (99 ± 18°). In contrast, the IR of the dominant shoulder (93 ± 17°) had significantly less ROM than the non-dominant shoulder (105 ± 17°) ($p<0.001$). Similarly the average total rotation (sum of IR and ER) of the dominant shoulder (198 ± 27°) and significantly less than the non-dominant shoulder (204 ± 29°) ($p<0.001$). This was a result of the larger difference in IR than ER ROM assessments for dominant and non-dominant shoulders.

Table 2.6. Range of motion (ROM) measures of dominant and non-dominant limbs.

	Mean ± SD	N	p-value [a]
SLR			
Dominant leg (mm)	89 ± 23	61	**0.012**
Non-dominant leg (mm)	84 ± 23		
Shoulder - IR			
Dominant arm (°)	93 ± 17	177	**<0.001**
Non-dominant arm (°)	105 ± 17		
Shoulder – ER			
Dominant arm (°)	105 ± 16	179	**<0.001**
Non-dominant arm (°)	99 ± 18		
Shoulder – TR			
Dominant arm (°)	198 ± 27	178	**<0.001**
Non-dominant arm (°)	204 ± 29		

Values are expressed as average ± standard deviation (SD). Significant differences are in bold.
[a] Dominant vs non-dominant limb (dependent t-test)
SLR - straight leg raise; IR - internal rotation; ER - external rotation; TR - total rotation; N - number of subjects; mm - millimeters.

The SLR sub-sample (N=51) were compared to the rest of the sample (N=241) for any differences in factors that were significantly associated with ROM measurements in the entire cohort (Table F.4 in Appendix F). There were no differences in the frequencies of gender or category of primary sports that were associated with a decreased lower body ROM. However, the SLR sub-sample

reported exercising in the 24 hours before exercise (17.2%) less frequently than the rest of the sample (37.8%) (p=0.028). Furthermore, the SLR sub-sample reported participating in sports that were associated with increased lower body ROM (17.1%) as their primary sport significantly more frequently than the rest (6.0%) of the sample (p=0.045).

Despite these significant differences in mean values, the dominant and non-dominant sides were significantly correlated for all of the ROM measures (Figure 2.3). It is important to note that the line of best fit (solid line) did not agree with the expected line of fit (dotted line), particularly with an increase in ROM. This deviation indicates that, in general, relatively hypermobile subjects exhibit greater differences in dominant versus non-dominant limb readings than normal subjects, or subjects with a reduced ROM in our cohort.

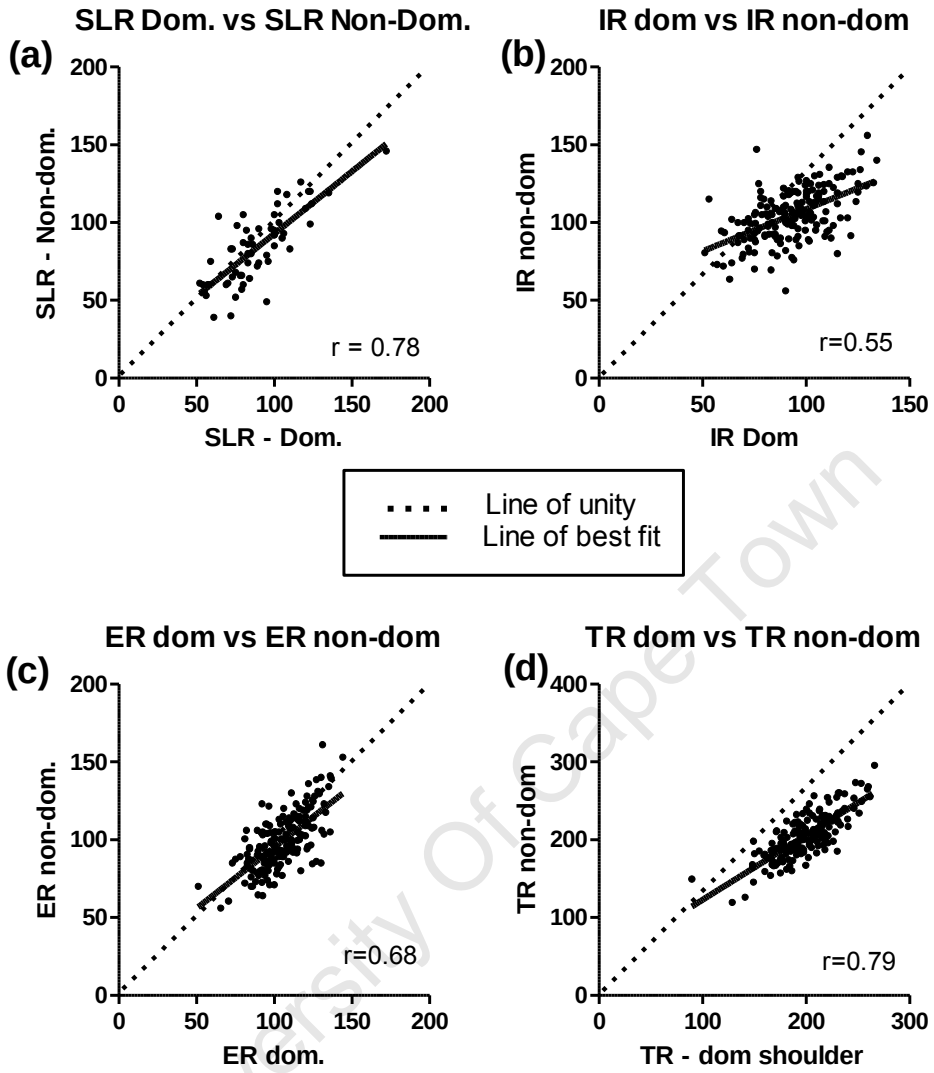

Figure 2.3. Range of motion (ROM) measurement correlations of (**a**) straight leg raise (SLR), (**b**) shoulder internal rotation (IR), (**c**) shoulder external rotation (ER) and (**d**) shoulder total rotation (TR) between the dominant (dom.) and non-dominant (non-dom.) sides. The solid line is the line of best fit while the dashed line is the line of unity.

2.3.4. Correlations between intrinsic/extrinsic factors and ROM measurements in males and females

As there were significant differences in the ROM measurements between the dominant and non-dominant limbs (Table 2.6) and since changes in ROM within the non-dominant limbs are less affected by participation in specific sports [27;28;49;78], ROM measurements within the non-dominant limbs were used for further analyses in this thesis. Furthermore, ShTR (ShIR + ShER) as opposed to ShIR or ShER, is less affected by sport participation [28] and this fact justified combining the two ROM measures for the non-dominant shoulder in Table 2.7 and 2.8. Therefore, the non dom. SLR, non-dom. ShTR and SR (a bilateral ROM assessment) will examined from this point forward, unless otherwise stated. The correlations, for males and females, of all the ROM measurements are, however, presented in Tables F.5 and F.6 in Appendix F.

There were no significant correlations with any of the intrinsic factors investigated (height, weight, BMI, waist circumference, age and flexibility training) and ROM measurements in males (Table 2.7) or females (Table 2.8).

For the subset of subjects recruited during the Two Oceans ultra-marathon, their finishing time (a proxy for performance), also did not significantly correlate with any ROM measurements in males (Table 2.7) or females (Table 2.8).

Table 2.7. Correlations between intrinsic and extrinsic factors and range of motion (ROM) measurements in males.

	SR	Non-dom SLR	Non-dom ShTR
Age	r=-0.04 N=199 p=0.603	r=-0.08 N=32 p=0.648	r=-0.11 N=102 p=0.282
Height	r=0.05 N=153 p=0.541	r=0.18 N=33 p=0.316	r=-0.16 N=102 p=0.113
Weight	r=-0.01 N=153 p=0.920	r=0.10 N=33 p=0.568	r=-0.15 N=102 p=0.132
BMI	r=-0.06 N=151 p=0.505	r=0.01 N=31 p=0.946	r=-0.10 N=100 p=0.330
Waist circumference	r=-0.02 N=86 p=0.858	r=0.07 N=17 p=0.782	r=-0.12 N=86 p=0.261
Flexibility training	r=0.12 N=151 p=0.136	r=0.31 N=30 p=0.096	r=-0.00 N=92 p=0.975
Two Oceans ultra-marathon finish time [a]	r=-0.09 N=61 p=0.472	n.d.	n.d.

[a] Only SR measurements were done on the Two Oceans ultra-marathon athletes.
TR – total rotation; dom – dominant; SR – sit and reach; SLR – straight leg raise; ShTR – shoulder total rotation; BMI – Body Mass Index; n.d. – not determined.

Table 2.8: Correlations between intrinsic and extrinsic factors and range of motion (ROM) measurements in females.

	SR	Non-dom SLR	Non-dom ShTR
Age	r=0.05 N=114 p=0.606	r=-0.33 N=26 p=0.095	r=-0.11 N=75 p=0.336
Height	r=-0.08 N=91 p=0.431	r=-0.32 N=26 p=0.108	r=-0.18 N=74 p=0.129
Weight	r=0.08 N=91 p=0.443	r=-0.28 N=26 p=0.163	r=0.05 N=74 p=0.692
BMI	r=0.15 N=91 p=0.161	r=-0.12 N=26 p=0.562	r=0.17 N=74 p=0.160
Waist circumference	r=-0.08 N=65 p=0.536	r=-0.23 N=20 p=0.322	r=0.16 N=68 p=0.196
Flexibility training	r=0.12 N=85 p=0.268	r=-0.01 N=22 p=0.967	r=-0.08 N=67 p=0.513
Two Oceans ultra-marathon finish time [a]	r=0.14 N=23 p=0.538	n.d.	n.d.

[a] Only SR measurements were done on the Two Oceans ultra-marathon athletes.
TR - total rotation; dom - dominant; SR - sit and reach; SLR - straight leg raise; ShTR - shoulder total rotation; BMI - Body Mass Index; n.d. - not determined.

In general, the ROM measurements increased with increasing BJHS score (Figure 2.4). Despite covarying for the effects of gender, SR ROM and non-dom. ShTR were significantly different between individual BJHS scores.

When grouped by the BJHS five-part questionnaire, “normal” subjects (score of less than two) had significantly lower ROM measurements when compared to the “hypermobile” subjects (score of two or more), despite covarying for the effects of gender (non-dominant SLR leg $p<0.05$; SR and non-dominant ShTR $p<0.001$) (Table 2.9).

Table 2.9. The range of motion (ROM) of normal (BJHS category <2) and hypermobile (BJHS category ≥2) of all the subjects.

	Normal	**Hypermobile**	**p-value** [a]
SR (mm)	240 ± 104 (164)	311 ± 103 (82)	**<0.001**
SLR – Non-dom. (°)	73.6 ± 18.6 (27)	93.1 ± 19.2 (20)	**0.012**
Shoulder T.R. – non-dom. (°)	196.2 ± 23.4 (104)	213.1 ± 31.6 (60)	**<0.001**

Values are expressed as average ± standard deviation with the number of subjects (N) in parentheses.
[a] Normal vs Hypermobile, p-value adjusted for effects of gender.

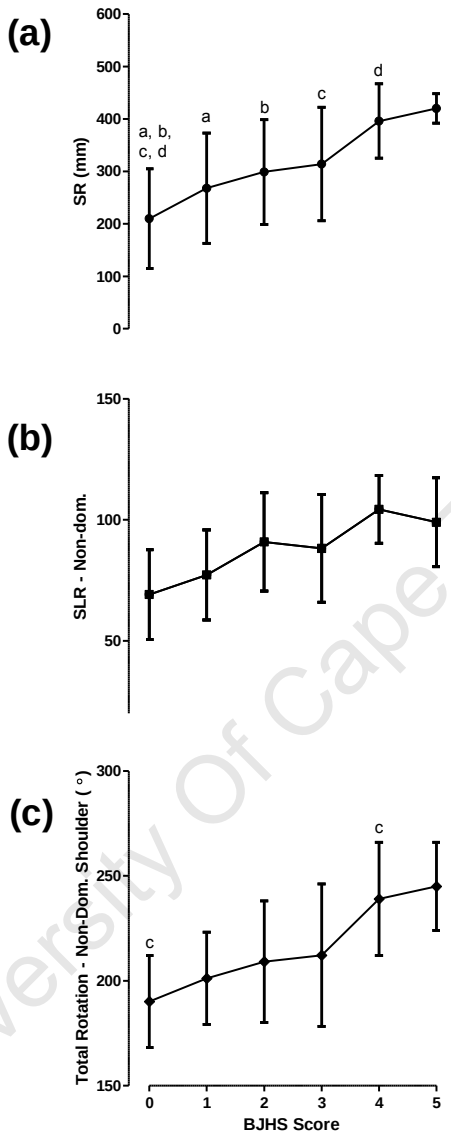

Figure 2.4. Plot of the mean (± standard deviation) ROM measures vs BJHS score. **(a)** SR, **(b)** Non-dom. SLR and **(c)** Non-dom. ShTR. The number of subjects (N) for each BJHS score is in parentheses.

Scheffe Pair-wise Post-hoc analyses (covaried for gender):

a: 0 vs 1 (p=0.01),

b: 0 vs 2, c: 0 vs 3, d: 0 vs 4, ($p<0.001$)

To analyse the effect of any reported non-serious injury on the ROM measurements, those who reported a non-serious injury were referred to as “currently injured” while those who did not were referred to as “uninjured”. A detailed inventory of the injuries is described in section 2.3.1. Owing to the even distribution of reported injuries to the right (42.3%, N=22) and left (40.4%, N=21) hand sides, both the dominant and non-dominant SLR measures were included in these analyses (Table 2.10). Furthermore, only lower body ROM measures were examined due to the high proportion of reported injuries (91%, N=61) having occurred to the lower body. As expected due to the fact that the reported injuries were “non-serious” in nature, when co-varied for gender, there were no significant differences in the lower body ROM measures for injured or uninjured subjects (Table 2.10).

In contrast, the participation in a certain categories of sport was significantly associated with altered ROM (Table 2.10). The categorisation of these sports was described previously in Section 2.3.2. Despite co-varying for gender, subjects that participated in sports that were associated with reduced ROM had significantly lower ROM for SR ($p<0.001$), dominant SLR ($p<0.05$), and non-dominant SLR ($p<0.05$) measures in comparison to those that participated in sports that were associated with an increased ROM.

Table 2.10. The effect of a self-reported current non-serious injury and sport participation on lower body range of motion (ROM) measurements.

	SR (mm)	**Dom SLR** (°)	**Non-dom SLR.** (°)
Current non-serious injury [a]			
No	266 ± 118 (188)	90.6 ± 24.9 (41)	84.9 ± 26.0 (41)
Yes [b]	259 ± 95 (65)	88.7 ± 22.7 (10)	84.0 ± 18.9 (10)
Sport participation, categorized by associated lower body ROM			
Reduced ROM	259 ± 103 (197) [d]	84.2 ± 24.7 (29) [c]	78.6 ± 25.2 (29) [c]
Increased ROM	351 ± 100 (17) [d]	114.5 ± 18.3 (6) [c]	108.8 ± 16.8 (6) [c]

Values are expressed as averages ± standard deviations, with the number of subjects (N) in parentheses.
p-values were co-varied for gender and the adjusted p-values reported.
[a] "Serious" classified as that requiring hospitalization or immobilization.
[b] Out of the 67 reported non-serious injuries, 91.0% (N=61) were to the lower body (lower back included in lower body). Of the 52 subjects that reported a side of injury, 42.3% (N=22) of injuries were to the Right Hand side, 40.4% (N=21) to the Left Hand Side and 17.3% (N=9) reported injury to both sides.
[c] Significantly different at $p<0.05$
[d] Significantly different at $p<0.001$
Dom – dominant; SLR - straight leg raise.

2.4 Discussion

The main finding of the study presented in this chapter was the general lack of associations between ROM measures and the common extrinsic and intrinsic factors associated with this trait in the literature (Section 1.6.1 and 1.6.2), which are summarised in Table 2.11.

Table 2.11. Extrinsic and intrinsic factors, along with the magnitude of certainty, associated with ROM. Whether an association or no association or the factor was not investigated is noted in the right column.

Intrinsic/ extrinsic	Factor	Certainty	Association/no association in cohort
Extrinsic	Level and type of activity	High	Association
	Temperature	Low	Did not investigate
Intrinsic	Age	High	No association
	Gender	High	Association
	Limb dominance	High	Association
	Flexibility training	High	No association
	Prior injury	Moderate	No association
	Weight/BMI	Moderate	No association
	Height	Low	No association
	Muscle size	Low	Did not investigate
	Ethnicity	Insufficient evidence	Did not investigate
	Genotype	Low	Did not investigate

The only extrinsic factor investigated in this study – level and type of sport participation – produced conflicting findings. While running performance in a marathon (proxy of performance) was specifically not correlated with SR ROM, generally the **type of sport activity** was associated with altered ROM measures.

As was expected, those who participated in a sport that was associated with reduced lower body ROM had significantly reduced SR and SLR values in comparison to those that participated in a sport associated with increased lower body ROM. The lack of association with running performance in this study was not entirely unexpected. While a single study [61] associated reduced ROM with better running economy, it should be acknowledged that this parameter is not the only determinant of performance [106]. While temperature was not specifically investigated in this study, it was recorded whenever possible during testing. During analyses ROM measures were always covaried for the effect of temperature, but it was never found to have a significant effect (data not shown) on ROM analyses.

Many intrinsic factors that have previously been associated with ROM (Section 1.6.2 and Table 2.11) were investigated concurrently in this study - namely, age, gender, height, weight/BMI, flexibility training, prior injury and limb dominance. Although not commonly reported to be associated with ROM, waist circumference was also analysed as an intrinsic factor for this dissertation. Ethnic differences in ROM were controlled for by only including white individuals in the study. Genotype will be discussed in greater detail in Chapter 3. Of the factors investigated in this study, only gender and limb dominance were significantly associated with ROM measures in our cohort. Females had significantly more ROM than males in all assessments, except in the non-dominant shoulder IR. This finding has been reported previously in a variety of age groups, populations

and measurements [21;28;66-68;72;73] (refer to Section 1.6.2.2) and is associated with ROM with a high level of certainty (Table 2.11), making this a confirmatory finding.

Similarly, the dominant limbs of both the upper and lower body had significantly different ROM to the non-dominant limb in the SLR and shoulder ROM assessments. Limb dominance is also associated with ROM with a high level of certainty (Table 2.11). However, the finding that the dominant leg was significantly more flexible than the non-dominant leg in our cohort is in contrast to the findings of Wang et al. [27]. This is noteworthy as Wang et al. [27] investigated a running population. While not specifically a running population, the majority of both the SLR sub-sample and the cohort in general reported running as their primary sport. In contrast, the difference in ROM measures in the IR and ER ROM of the shoulder is confirmatory of findings in both athletic [28] and non-athletic [66;77] populations. However, the difference in TR ROM between dominant and non-dominant shoulders was unexpected, even for a physically active population [28], but may be explained by the inaccuracy of this measurement (Section 2.2.8 - repeatability of methods).

However, the finding that increasing age was not associated with reduced ROM (associated with a high level of certainty - Table 2.11) in this cohort is in contrast to most reports in the literature [64;65] (Section 1.6.2.1). Two prominent theories have been proposed to explain this association (Chapter 1, Section 1.6.2.1). One

theory postulates that the reduction in ROM is a factor of the concurrent reduction in physical activity with increasing age [9]. The alternate theory focuses at a molecular level, explaining the decline in ROM as a result mainly of biochemical changes to the extracellular matrix with increasing age [69]. While this particular study did not investigate the molecular nature of the cohort, the physical activity of the sample was ascertained indirectly. The majority of subjects (96.4%), recruited outside of running events, reported at least one current sport in the medical questionnaire (Appendix D2). Furthermore, this sub-cohort reported an average of 8.6 hours of sport training per week (range 1-39 hours/week) at the time of testing (this factor is explored in greater detail in Chapter 3). While the running sub-cohort were not asked these two questions in their questionnaire directly, the fact that they were recruited from ultra-marathon and marathon running events implies that they too were physically active. These findings confirm that this cohort, in general, was indeed "physically active" and therefore may explain the lack of association of ROM with age. While the majority of subjects in this cohort (approximately 50%) were students from nearby universities, younger than 35 years of age, our entire cohort was fairly homogenous for reported physical activity (data shown in Chapter 3). The students were also not significantly different to the rest of the cohort in terms of anthropometric data or the percentage of each day that was spent sitting.

A fairly high proportion of the sample (47.1%) reported a previous (>24 months before testing) connective tissue injury/disorder. However, this finding is

confirmatory of a physically active population, which are at high risk of developing musculoskeletal soft-tissue injury [38;107;108]. ROM measurements were not significantly different for those who reported a history of injury or those who did not (data not shown).

As expected in a physically active population, those that reported a current non-serious injury were significantly older than those who did not [49;51]. While prior injury has been associated with reduced ROM previously [37;59;79], there was no difference in ROM between "injured" or "uninjured" individuals in our cohort (Section 2.3.1). However, the strict exclusion criteria for injury in this study (an incident to musculoskeletal tissue requiring hospitalization or immobilization in the past 24 months) could account for this lack of difference. The fact that these injuries were "non-serious" (i.e. not requiring hospitalization or immobilization) could also explain the diversity, and at times, inaccuracies of reported injuries (Appendix F2, Table F.1 and F.2) in our cohort.

The lack of association between flexibility training and ROM measures is in contrast to findings of many studies investigating this factor [25;86;88-91]. Although less than half of the cohort reported performing regular flexibility training (stretching), this factor is associated with increased ROM with a high level of certainty (Table 2.11), making the lack of association in this cohort surprising. Another potential explanation for this lack of association is the theory (author's view - unsupported) that this factor is individually mediated. Similarly to the

molecular theory of the response of ROM to aging, this theory could only be investigated with a concurrent molecular investigation of all of these factors. This will be discussed in the introduction to Chapter 3 (Section 3.1). Furthermore, the recording of flexibility training, from the self-reported medical questionnaire, was not a well controlled measure of this factor.

Furthermore, there were no associations between anthropometric measures - weight/BMI, height, and waist circumference - and ROM in our cohort. While there is was no literature available on waist circumference, weight/BMI [81;82] and height [83] have both been associated with reduced ROM previously in the literature (Section 1.6.2). However, the very narrow range of these variables the cohort may explain the lack of association.

In terms of the ROM assessment techniques of this study, the lower body ROM measures were all very reliable (Section 2.2.8). The sit and reach measurement was also very well correlated with the straight leg raise assessments as should be expected from these two assessments (Appendix F, Table F.7) [104].

Large cohort studies in young adults noted have reported a hypermobility prevalence of between 2-35% for males and 5-57% for females [1] confirmatory of the prevalence of 24.8% in males and 45.9% in females in our cohort. Furthermore, the BJHS score was positively associated with SR ROM. With further research in this area, this score may provide clinicians with a predictive

reference point for ROM assessments in patients. While the small sample sizes reporting 4 or more affirmative answers to this questionnaire reduced statistical power for this analysis, these low prevalence's would be expected in an apparently healthy population such as ours.

The major limitation of this study was the non-normal age distribution and homogeneity of the population. The majority of the 325 recruited subjects were students from the University of Cape Town, between the ages of 20 and 25 years (34%, N=109). However, this was probably a factor of the inclusion and exclusion criteria of this study required that subjects to be apparently healthy and physically active - both of which were confirmed. While unintentional, this homogeneity proved to be an essential factor for the following chapter (Chapter 3). Another limitation of the study was the fact that the testing temperature was not kept consistent for all testing sessions. However, the temperature was noted whenever possible and ROM measurements were also covaried for this factor. The fact that the population was such an active population introduced a couple of potentially confounding variables, one of which was found to be significant. Specifically, despite being asked to avoid unaccustomed exercise in the 24 hours before being tested, a number of subjects noted some form of exercise during this period immediately prior to testing. Interestingly, those that reported having exercised in the 24 prior to testing had significantly reduced SR ROM than those who did not report partaking in any exercise. While this finding is not understood, it should not be ignored and will be explored further in Chapter 3.

Another limitation of the study was the classification of sports (Appendix F3). While literature was consulted wherever possible to categorize sports by their association with lower body ROM, the diversity of listed sports made this process difficult and open to error of over-simplification. Furthermore, the system used to classify the studies investigating intrinsic and extrinsic factors associated with ROM (magnitude of net benefit [44]) was largely subjective. This was due to the fact that this system was originally intended to assess and compare risk factors for injury and illness, but was adapted for this thesis for cross-sectional studies investigating the association of various factors with ROM.

In conclusion, there was a general lack of association with intrinsic and extrinsic factors associated with ROM measures in an apparently healthy and physically active population. Of the commonly associated intrinsic and extrinsic factors, only gender, limb dominance and type of sport were found to be significantly related to ROM measures. These factors have all been associated with ROM with a HIGH level of certainty previously (Table 2.11). While the lack of association with anthropometric variables may be explained by the relative homogeneity of the cohort, the lack of association with two factors - age and flexibility training is noteworthy. In particular, these two factors are associated with ROM with a high level of certainty (Table 2.11). A variable, unaccounted for up until this point, that could explain both of these findings is genotype-mediated response of both aging

and flexibility training on ROM. This will be the focus of the next chapter (Chapter 2).

Chapter 3

The association between sequence variants within the *COL5A1* gene and range of motion (ROM) measurements in an apparently healthy and physically active population.

3.1 Introduction

In Chapter 2, it was discovered that relatively few of intrinsic and extrinsic factors commonly associated with ROM were associated with an apparently healthy and physically active cohort. The lack of association was particularly surprising of two factors, age and flexibility training, that are associated with ROM with a high level of certainty (Table 2.11). Genetic sequence variants have been associated with phenotypic variation in the sports medicine sphere previously [94]. Genetic mutations have been shown to cause HDCTs, which present with a common clinical feature of joint hypermobility [1]. A number of genes that encode for extracellular proteins of connective tissue have exhibited mutations in sufferers of HDCTs [1;30]. In fact, over half the patients who present with classic Ehlers-Danlos Syndrome (EDS), a common HDCT, possess a disease-causing mutation in the

COL5A1 gene [30]. ROM is a heritable trait, with its heritability estimated to be between 64% [92] and 70% [93] in classical twin studies. Furthermore, it has been suggested that genetic sequence variants are associated with ROM in healthy populations [32]. However, evidence of this association has only been produced in a single cohort, which was comprised mainly of injured individuals [97].

Therefore, the aim of the study presented in this chapter is to investigate whether two SNPs, the *Bst*UI and *Dpn*II RFLPs, within the 3'UTR region of the *COL5A1* gene were associated with ROM measurements in an apparently healthy and physically active cohort.

3.2 Methods

3.2.1 Subject recruitment and testing procedure

Subject recruitment, pre-testing procedure, anthropometric assessments and ROM assessments were performed as previously described in Section 2.2 and Figures 1 and 2 of Chapter 2. Only white subjects were genotyped and analysed due to the potentially confounding effect of population stratification between different ethnic groups in genetic investigations [109]. Subjects were genotyped for two different single nucleotide polymorphisms (SNPs) in the *COL5A1* gene using the methods detailed below in sections 3.2.2 and 3.2.3. A total of 302 subjects

(187 males and 115 females) were genotyped for the *COL5A1* BstUI RFLP (SNP rs12722) (Table 3.1). For the DpnII RFLP (SNP rs13946), a total of 298 subjects (181 males and 113 females) were genotyped (Table 3.2).

3.2.2 Blood collection and DNA extraction

Five milliliters (ml) of venous blood was obtained from each subject by venipuncture of an antecubital fossa vein and collected in an ethylenediaminetetraacetic (EDTA) Vacutainer. The sample was frozen and stored at -20°C until DNA extraction. The DNA extraction was performed as described by Lahiri and Nurenberg [110] with slight modifications (Appendix G). Briefly, the blood samples were transferred to polypropylene tubes and 10ml TKM1 buffer (10 mM Tris-HCL pH 7.6, 10 mM KCl, 10 mM $MgCl_2$ and 2 mM EDTA), containing 2.5% Nonidet P-40, was added to lyse the red blood cells. The solution was allowed to incubate at room temperature for 10 minutes, after which it was centrifuged at 3000 rpm (1200 Xg) for 10 minutes at room temperature in order to pellet the white blood cells. Eight hundred ml of TKM2 buffer (10 mM Tris-HCL pH 7.6, 10 mM KCl, 10 mM $MgCl_2$, 0.4 M $NaCl_2$ and 2 mM EDTA), containing 50 µL of 10% sodium dodecyl sulphate (SDS), was then added and the solution incubated at 55°C for 60 minutes, or until the pellets had dissolved. One hundred and fifty microlitres of $NaClO_4$ and 500 µL of molecular grade chloroform were added, and the mixture was vortexed thoroughly. The

suspension was then transferred to 1.5 ml microfuge tubes before centrifuging at 13000 r.p.m (1200Xg) for 10 minutes at room temperature. Five hundred microlitres of the top aqueous phase was then transferred to a new sterile 1.5 ml microfuge tube. One ml of 100% ethanol was added to this aqueous phase and the solution centrifuged at 13000 r.p.m (1200Xg) for five minutes at room temperature. With the DNA precipitated out, the tubes were opened, inverted and allowed to air dry for 2-3 hours. Finally, 200 µL of 1xTE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was added to the precipitate and the tubes incubated at 65°C for 15 minutes. This DNA solution was stored at 4°C until genotyping analysis was performed (Section 3.2.3).

3.2.3 *COL5A1* genotyping

A 667 base pair (bp) DNA, containing the *Bst*UI (SNP rs12722) and *Dpn*II (SNP rs13946) RFLP was amplified using a polymerase chain reaction (PCR) as described by Greenspan and Pasquinelli [111]. The following primers were used to amplify the 3'-untranslated region (UTR) of the *COL5A1* gene: Forward (COL5F): 5' GAA GAC GGT TCT GGA GAT CG 3'; Reverse (COL5R): GAA GGC ACC TGC AGA ATG AC 3'. A diagram representing the relative positions of these two SNPs within the 3'-UTR of the *COL5A1* gene are presented in Figure 3.1. The C and T alleles of the two SNPs were identified using either the *Bst*UI or *Dpn*II restriction endonuclease enzymes.

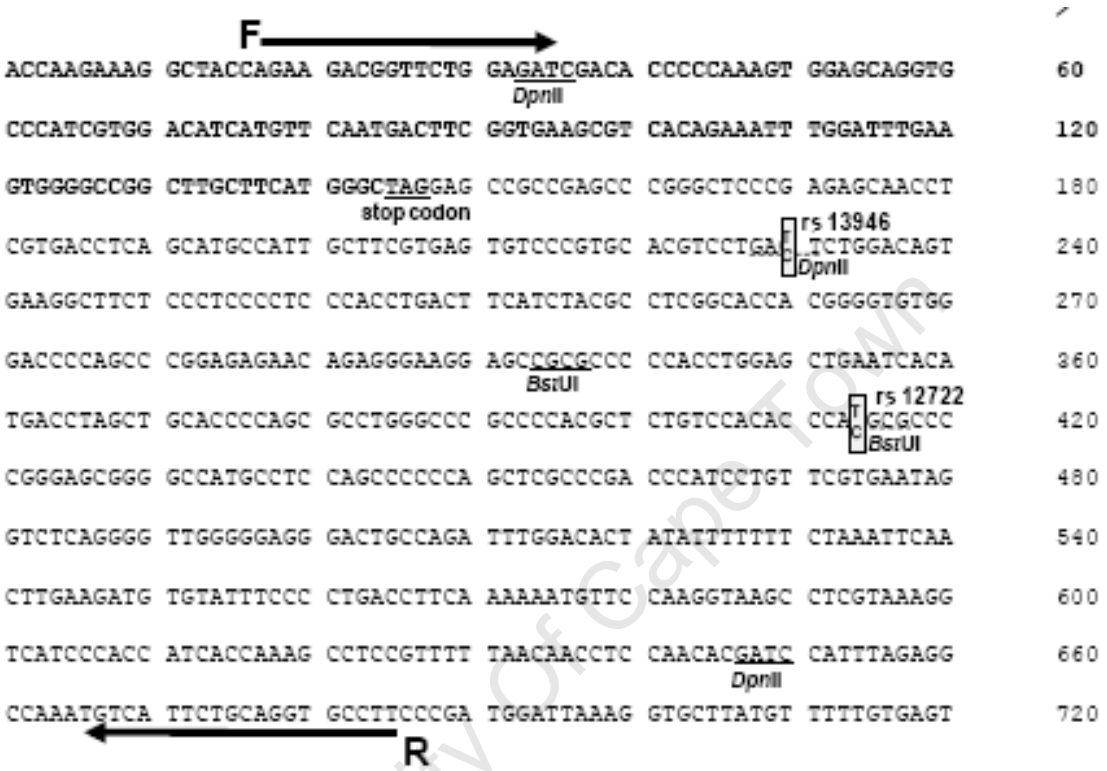

Figure 3.1 [112] The 720 base pair (bp) genomic sequence at the 3'-UTR (untranslated region) of the *COL5A1* gene. The binding position of the forward (F) and reverse (R) primers (solid arrows) designed to amplify a 667 bp PCR fragment containing the *Dpn*II (single nucleotide polymorphism, SNP rs13946) and *Bst*UI (SNP rs12722) restriction fragment length polymorphisms (RFLPs) are indicated. The recognition (underlined) sequences of the *Dpn*II (/GATC) and *Bst*UI (CG/CG) restriction enzymes are also indicated on the genomic sequence. The T to C substitutions (boxed) for the *Bst*UI and *Dpn*II RFLPs cause a recognition sequence to be destroyed.

The PCR was carried out in a total volume of 60 µl containing: at least 100 ng of DNA, 20 pmol of forward and reverse primers, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCL_2$, 0.2 mM of each dNTP (dATP, dTTP, dCTP and dGTP) and 2.3 U of DNA Taq Polymerase. A PCR Express Thermal Cycler (Hybaid Limited, Middlesex, UK) was used to perform the amplification using a pre-programmed process. The process was performed with an initial denaturing step (94°C for 3min), followed by 35 cycles of denaturing (94°C for 1min), an annealing step (53°C for 1min), an extension step (75°C for 1min) and a final extension step (72°C for 8min). The PCR product was digested with *Dpn*II and *Bst*UI endonuclease enzymes. Digestion with the *Dpn*II enzyme yielded fragments of 418, 194, 40 and 15 bp for the T allele and 612, 40 and 15 bp for the C allele. Digestion with the *Bst*UI restriction enzyme yielded fragments of 351 and 316 bp for the T allele and 316, 271 and 80 bp for the C allele. Three genotypes are therefore possible in these two RFLPs, namely TT, TC or CC (Figures 3.2 and 3.3).

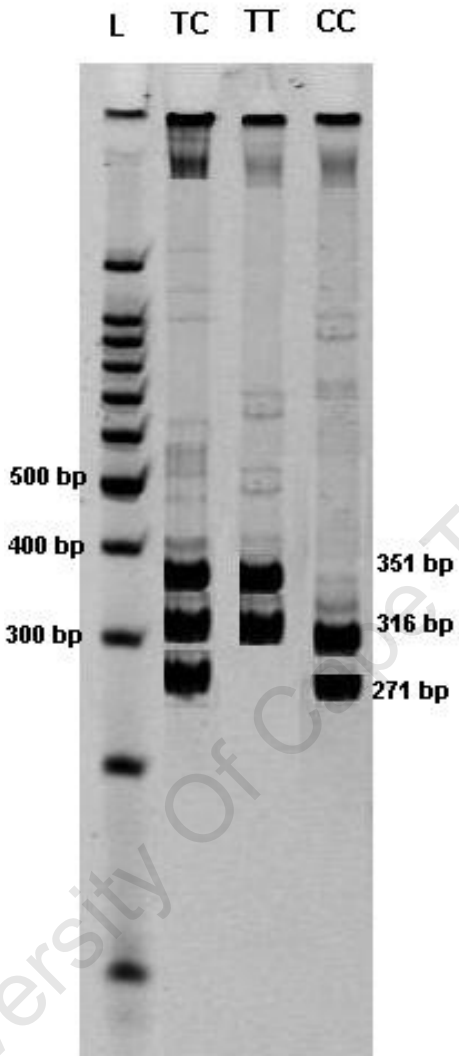

Figure 3.2. A typical 6% non-denaturing polyacrylamide gel showing the three genotypes (CC, TC and TT) of the *COL5A1 Bst*UI restriction fragment length polymorphism (RFLP). Digestion of the 667 base pair (bp) PCR product with *Bst*UI produced 351 bp and 316 bp fragments for the T allele, and 316 bp, 271 bp and 80 bp fragments for the C allele. The 80 bp fragment ran off the gel and is therefore not visible. The left lane contains the 100 bp molecular weight ladder (L) with the appropriate fragment sizes given in bp. The appropriate bp sizes for the T and C allele fragments are noted on the right hand side.

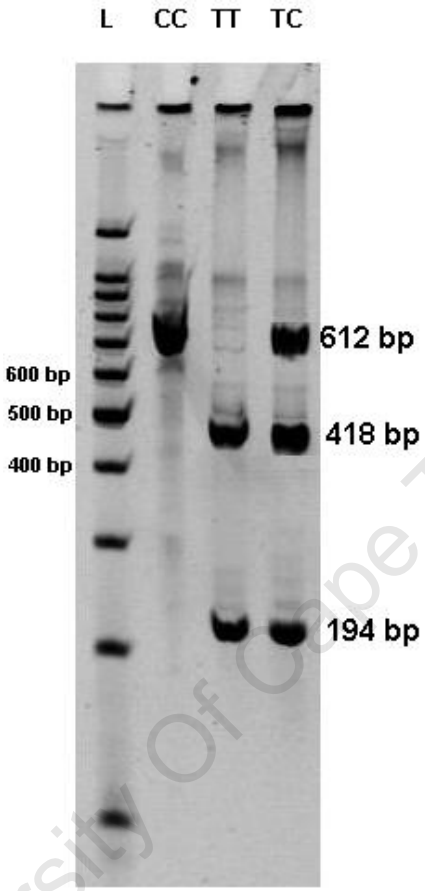

Figure 3.3. A typical 6% non-denaturing polyacrylamide gel showing the genotypes (TC, TT and TC) of the *COL5A1 Dpn*II restriction fragment length polymorphism (RFLP). Digestion of the 667 base pair (bp) PCR product with the restriction enzyme, *Dpn*II, produced 612 bp, 40 bp and 15 bp fragments for the C allele and 418 bp, 194 bp, 40 bp and 15 bp fragments for the T allele. The 40 bp and 15 bp fragments ran off the gel and are therefore not visible. The left lane contains the 100 bp molecular weight ladder (L) with the appropriate fragment sizes given in bp. The appropriate bp sizes for the T and C allele fragments are noted on the right hand side.

The resulting fragments, as well as *SYBER® Gold* nucleic acid gel stain (*Invitrogen Molecular Probes*™, Oregon, USA), were separated on 6% non-denaturing polyacrylamide. A 100 bp DNA ladder (Promega Corporation, Madison, Wisconsin, USA), with known size markers was loaded on each gel as a reference point. Furthermore an "uncut" product of the PCR process was loaded for the same reason. Once separated at 140 V for 2 hours, the gels were photographed under ultraviolet (U.V.) light using a Uvitec photodocumentation system (Uvitec limited, Cambridge, UK) and the DNA fragment sizes identified using the DNA ladder as a reference. These fragment sizes indicated which particular genotype the individual possessed for both RFLPs. The subject was only identified by a blood sample number on the data sheet and EDTA Vacutainer in the interests of their complete anonymity.

3.2.4 Statistical analyses

General descriptive statistics were performed on the entire sample, divided into the three *COL5A1* genotype groups for both of the SNPs (TT, TC or CC). Levene's tests of homogeneity were performed to test for differences in homogeneity of the data. Analysis of variance tests (ANOVA) were then used to examine differences between categorical data (genotype group, age category) and continuous data (age, height, weight, SR score). Chi-squared tests were used to examine differences between two sets of categorical data. Analysis of co-variance (ANCOVA) tests were used to remove factors that could possible

confound these findings. These factors were dependent on well-known differences previously published in the literature (gender) and differences specific to this sample (e.g. waist circumference and weight). The SR ROM measurements were used to divide the cohort into three tertiles, High, Intermediate (Int.) and Low ROM, to assess the frequency of genotype distributions within these tertiles. This was done in order to gain a visual representation and assess if any linear trends existed for any of the genotypes. Multiple regression analyses were used to test the interaction between genotype and various intrinsic factors as were detailed in the previous chapter. Based on the findings of the finding of a significant interaction between SR ROM and age in the *Bst*UI RFLP, the sample was divided into "young" or "old" age groups, with 18 – 34 years of age being considered "young" and ≥35 years being considered "old". Analyses described above (descriptive, Levene's, ANOVA, chi-squared and ANCOVA) were repeated on these two age categories. A multivariate analysis with forward stepwise regression was used to determine the model that best predicted SR ROM with factors that had been significantly associated with ROM measures in Chapter 2 and Chapter 3 for the "old" group.

GraphPad Prism Version 5.02 was used for drawing the frequency of genotypes within *Bst*UI and *Dpn*II RFLPs. Linear regression lines were applied to determine the r-value (slope of the line) of SR scores with increasing age and amount of reported flexibility training for *Bst*UI and *Dpn*II RFLP genotypes.

Hardy-Weinberg equilibrium (HWE) was assessed using Hardy-Weinberg Exact Tests (http://genepop.curtin.edu.au - Genepop version 4.0.10) on both RFLPs in order to assess if the cohort were genetically homogenous (Appendix H1 and H2). Both the *Bst*UI (p=0.555) and *Dpn*II (p=0.774) RFLPs were in HWE for this cohort.

3.3 Results

3.3.1 General characteristics of the two *COL5A1* single nucleotide polymorphisms (SNPs): *Bst*UI (SNP rs12722) and *Dpn*II (SNP rs13946) RFLPs.

The genotype frequencies of the SNPs within the *COL5A1* gene were: *Bst*UI RFLP: 33.1% TT (N=100), 47.4% TC (N=143) and 19.5% CC (N=59); and *Dpn*II RFLP: 51.7% TT (N=154), 39.9% TC (N=119) and 8.3% CC (N=25). The genotype distributions for both RFLPs were in Hardy-Weinberg equilibrium (*Bst*UI RFLP =0.555 and *Dpn*II RFLP = 0.774).

Although there were no significant differences in the gender frequencies between the three *Bst*UI RFLP genotype groups (Table 3.1), there were relatively more males in the TT genotype than either the TC or CC genotypes. Therefore, all further analyses were therefore adjusted for gender, when appropriate. After adjusting for gender, the three *Bst*UI RFLP genotype groups were matched for

age, height, weight and body mass index (BMI). They were also matched for the frequency of subjects who reported exercising in the 24 hours prior to testing (shown to have a significant influence on SR measurements in Chapter 2). Furthermore, the amount of reported training per week at the time of testing was not significantly different between genotype groups. However, waist circumference was still significantly different ($p=0.009$) between genotype groups, even after accounting for the effect of gender. The TT genotype group had, on average, a significantly larger waist circumference than the TT or CC groups. The three genotype groups were also matched for occupation, limb dominance, hypermobility, current non-serious injury and history of connective tissue injury/pathology (Table 3.1 and data not shown).

Table 3.1. Descriptive data of the three BstUI RFLP (SNP rs12722) genotype groups.

	TT	TC	CC	*p*-value [a]
Gender (% males)	68.0 (100)	61.6 (143)	52.5 (59)	0.151
Age (years)	30.3 ± 10.2 (100)	32.0 ± 11.3 (142)	32.7 ± 11.6 (59)	0.329
Height (m)	1.76 ± 0.09 (86)	1.75 ± 0.09 (112)	1.73 ± 0.10 (46)	0.428 [b]
Weight (kg)	76.2 ± 13.8 (86)	72.3 ± 12.8 (112)	69.8 ± 11.2 (46)	0.060 [b]
BMI (kg/m^2)	24.3 ± 3.1 (85)	23.5 ± 2.8 (111)	23.3 ± 2.3 (46)	0.154 [b]
Waist circumference (cm)	80.4 ± 9.7 (54) [‡, †]	77.0 ± 7.3 (74) [‡]	75.3 ± 6.7 (28) [†]	**0.009** [b]
Flex. training (min/wk)	1.04 ± 1.84 (79)	1.26 ± 2.57 (109)	1.94 ± 4.1 (44)	0.212
Ex. past 24 hrs before visit 1 (% "Yes")	35.2 (91)	36.2 (127)	35.7 (56)	0.987
Students (%)	49.3 (75)	42.9 (98)	37.8 (37)	0.477
Current training (hr/wk)	8.3 ± 6.1 (52)	9.4 ± 7.0 (59)	7.2 ± 4.9 (24)	0.339
Hypermobile (% "Yes")	35.4 (79)	29.4 (109)	41.2 (51)	0.248
Current injury (% "Yes") [c]	19.0 (84)	29.6 (115)	22.9 (48)	0.224
CT injury/disorder (% "Yes") [d]	51.2 (84)	47.0 (115)	45.8 (48)	0.787

Values are expressed as average ± standard deviation or as a frequency. The number of subjects (N) is in parentheses. Age, height, weight and BJHS score and limb dominance were obtained or measured during the first visit. Body mass index (BMI) was calculated as kilograms per meter squared. Occupation, training hours and Injury data were self-reported in a questionnaire.

[a] TT vs TC vs CC.

[b] p-value adjusted for gender.

[c] non-serious (did not require hospitalization or immobilization).

[d] previous history of either a connective tissue injury or disorder

Post-hoc analyses (Scheffe Test): [‡] TT vs TC, p=0.014, [†] TT vs CC, p=0.004 and

SNP - single nucleotide polymorphism; RFLP - restriction fragment length polymorphism; m - meter; kg - kilogram; cm - centimeters; min - minutes; CT - connective tissue; Flex – flexibility; wk - week; Ex. – exercise; hr - hour.

For the *Dpn*II RFLP (Table 3.2), there was also a noticeable, although not significant, difference in gender frequency between the three genotypes. After covarying for gender, weight (p=0.039) and waist circumference (p=0.029) were still significantly different between the genotypes. For both of these measures the TT group displayed on average, significantly larger values than the TC group. Besides these two variables, the three groups were matched for all other descriptive data, including exercise in the 24 hours before visit 1 and training hours per week. A Levene's test determined that results for BMI were not homogeneous and therefore should be interpreted with caution.

3.3.2 ROM measurements between the three genotype groups of the two *COL5A1* SNPs: *Bst*UI and *Dpn*II RFLPs.

For consistency, the same ROM measures were examined in this chapter as in the previous chapter (Chapter 2). For reasons described above in section 3.3.1 the ROM measures were adjusted for gender. Waist circumference and weight were different (significantly or a trend) between genotype groups (Tables 3.1 and 3.2), and these two anthropometric variables are also closely correlated in our cohort (r=0.88, N=156, p<0.001). Therefore, ROM measures within the *Bst*UI and *Dpn*II RFLP groups were only covaried for weight, for which there was more available data.

Table 3.2. General characteristics of the three *Dpn*II RFLP (SNP rs13946) genotype groups.

	TT	TC	CC	*p*-value [a]
Gender (% males)	64.3 (154)	57.1 (119)	64.0 (25)	0.467
Age (years)	31.3 ± 11.4 (151)	31.2 ± 10.3 (119)	34.2 ± 12.6 (19)	0.427
Height (m)	1.76 ± 0.09 (125)	1.73 ± 0.09 (95)	1.76 ± 0.08 (18)	0.225 [b]
Weight (kg)	75.0 ± 13.5 (125)	70.5 ± 12.7 (95)	72.6 ± 10.6 (18)	**0.039** [b, c]
BMI (kg/m^2)	24.2 ± 3.2 (123)	23.3 ± 2.6 (95)	23.2 ± 2.1 (118)	0.094 [b, f]
Waist circumference (cm)	79.3 ± 9.2 (87)	75.8 ± 6.5 (60)	77.1 ± 8.4 (8)	**0.029** [b, c]
Flex. training (min/wk)	1.20 ± 2.8 (119)	1.52 ± 2.83 (91)	1.04 ± 1.4 (17)	0.645
Ex. past 24 hrs before visit 1 (% "Yes")	35.0 (140)	40.6 (106)	21.7 (23)	0.218
Students (%)	50.5 (107)	40.7 (81)	27.8 (18)	0.163
Current training (hr/week)	8.8 ± 5.6 (73)	8.5 ± 7.3 (59)	8.1 ± 6.0 (9)	0.925
Hypermobile (% "Yes")	34.8 (115)	35.4 (99)	21.1 (19)	0.131
Current injury (% "Yes") [d]	25.4 (126)	27.1 (96)	10.0 (20)	0.267
CT injury/disorder (% "Yes") [e]	50.0 (126)	43.8 (96)	55.0 (20)	0.525

Values are expressed as average ± standard deviation or as a frequency. The number of subjects (N) is in parentheses. Age, height, weight and BJHS score and limb dominance were obtained or measured during the first visit. Body mass index (BMI) was calculated as kilograms per meter squared. Occupation, training hours and Injury data were self-reported in a questionnaire.

[a] TT vs TC vs CC,

[b] p-value adjusted for gender

[c] Post-hoc analyses (Scheffe Test): TT vs TC ($p<0.05$)

[d] non-serious (did not require hospitalization or immobilization).

[e] previous history of either a connective tissue injury or disorder

[f] Levene's test of homogeneity was significantly different.

SNP - single nucleotide polymorphism; RFLP - restriction fragment length polymorphism; m - meter; kg - kilogram; cm - centimeters; min - minutes; CT - connective tissue; Flex. - flexibility; wk - week; Ex. - exercise; hr - hour.

After covarying for gender and weight, neither the *Bst*UI RFLP nor the *Dpn*II RFLP displayed any significant differences for any of the ROM measures (Table 3.3).

Table 3.3 Comparison of the range of motion (ROM) measurements between the *Bst*UI and *Dpn*II RFLP genotype groups.

	TT	TC	CC	p-value [a]
BstUI RFLP Genotype				
SR (mm)	261 ± 108 (99)	256 ± 103 (136)	288 ± 119 (59)	0.508 [b]
Non-dom SLR (°)	79 ± 22 (17)	88 ± 24 (32)	84 ± 25 (10)	0.457 [b]
Non-dom ShTR (°)	201 ± 27 (59)	206 ± 29 (87)	204 ± 31 (32)	0.585 [b]
DpnII RFLP Genotype				
SR (mm)	256 ± 112 (150)	266 ± 104 (114)	301 ± 110 (25)	0.289 [c]
Non-dom SLR (°)	82 ± 22 (30)	89 ± 22 (25)	79 ± 36 (4)	0.526 [c]
Non-dom ShTR (°)	203 ± 28 (96)	208 ± 30 (70)	193 ± 32 (11)	0.626 [c]

Values are expressed as average ± standard deviation, with the number of subjects (N) in parentheses.
[a] TT vs TC vs CC
[b] p-value adjusted for gender and weight
[c] p-value adjusted for gender and waist circumference
RFLP - restriction fragment length polymorphism; SR - sit and reach; Non-dom - non dominant; SLR - straight leg raise; ShTR - Shoulder total rotation.

3.3.3 Differences in *COL5A1* genotype frequencies within the High, Intermediate and Low ROM tertile groups

To facilitate comparison between ROM measures in the *Bst*UI and *Dpn*II genotypes, subjects were divided into ROM tertiles for SR scores. Data were analysed in this fashion in order to visualise the frequency distribution of genotypes within this tertiles and also to investigate any possible linear trends among the genotypes. As expected there were significant differences in the distribution of males and females between SR tertiles, with the most and least females in the High and Low SR ROM tertile groups, respectively (Table 3.4). Similarly, there were more and less clinically hypermobile subjects in the High and Low SR ROM tertiles, respectively. Furthermore, there were more subjects who reported a current, non-serious injury in the intermediate SR tertile than in the high and low tertiles. While not significantly different, more flexibility training was reported to be performed by the subjects in the intermediate, than the High or Low ROM tertiles. There were no other significant differences in any of the other characteristics between the SR ROM tertiles.

Table 3.4. General characteristics of the SR ROM tertile groups.

	High ROM	Intermediate ROM	Low ROM	p-value [a]
Gender (% Male)	41.0 (105)	71.4 (105)	77.1 (105)	**<0.001**
Age (years)	31.1 ± 10.0 (105)	32.5 ± 11.3 (103)	32.8 ± 11.8 (105)	0.480
Height (m)	1.71 ± 0.10 (89)	1.77 ± 0.08 (75)	1.75 ± 0.08 (80)	0.798[b]
Weight (kg)	68.9 ± 11.9 (89)	76.2 ± 12.9 (75)	74.8 ± 12.9 (80)	0.737 [b]
BMI (kg/m^2)	23.3 ± 2.5 (88)	24.1 ± 3.0 (75)	23.8 ± 2.9 (79)	0.810 [b]
Waist circumference (cm)	75.1 ± 7.6 (56)	75.1 ± 7.6 (56)	75.1 ± 7.6 (56)	0.394 [b]
Flex. training (min/wk)	1.62 ± 3.57 (84)	2.22 ± 4.87 (75)	0.75 ± 2.18 (77)	0.050
Hypermobile (% "Yes")	51.8 (83)	27.2 (81)	20.7 (82)	**<0.001**
Ex. past 24 hrs before visit 1 (% "Yes")	29.3 (99)	37.9 (95)	41.8 (98)	0.173
Current injury [c] (% "Yes")	16.7 (90)	36.3 (80)	25.3 (83)	**0.014**

Values are expressed as average ± standard deviation or as a frequency. The number of subjects (N) is in parentheses. Gender, age, height, weight and hypermobility (as determined from the benign joint hypermobility score), waist circumference and exercise (Ex) participation were obtained or measured during the first visit. Body mass index (BMI) was calculated as kilograms per meter squared. Flexibility (Flex.) training and injury data were self-reported in a questionnaire.
[a] TT vs TC vs CC.
[b] p-value adjusted for gender.
[c] non-serious (did not require hospitalization or immobilization).
m - meter; kg - kilogram; cm - centimeters; min - minutes; wk - week.

Owing to the similar frequency distribution of SR tertiles within the TT and TC genotypes, and to increase statistical power, these were combined for analysis against the CC genotype. Furthermore, due to low sample numbers in the CC genotype the Int. and Low SR ROM tertiles were combined for analysis against the High SR ROM tertile. There were no significant differences in the *COL5A1 Bst*UI (Figure 3.4) and *Dpn*II (Figure 3.5) RFLP genotype distributions between the High, Intermediate and Low SR ROM tertiles. The *COL5A1 Bst*UI RFLP CC genotype was, however, significantly over-represented (p=0.031) in the high ROM group (CC genotype: 47.5%, N=28 and T allele: 30.2%, N=71) when compared to the combined Intermediate and Low ROM groups (CC genotype: 52.5%, N=31 and T allele: 69.8%, N=164) (Figure 3.4). In addition, there was a significant linear trend (p=0.044) when the *COL5A1* DpnII RFLP CC genotype was compared to the T allele (TT + TC genotypes) (Figure 3.5) in that the CC genotype decreased from 13.3% in the High to 5.2% in the Low ROM tertile. Despite dividing the non-dominant SLR and non-dominant ShTR ROM measures into halves, low sample numbers meant that no meaningful analyses of the measures could be reported (Table H.1 and H.2 in Appendix H3)

Furthermore, we explored the effect of genotype on ROM by diving the cohort into “normal” and “hypermobile” by the BJHS score classification (Figures 3.6a and 3.6b, respectively) [33]. In contrast to the findings for the SR ROM tertiles, there were no significant differences in the *COL5A1 Bst*UI or *Dpn*II RFLPs genotype distributions when the subjects were divided by this method.

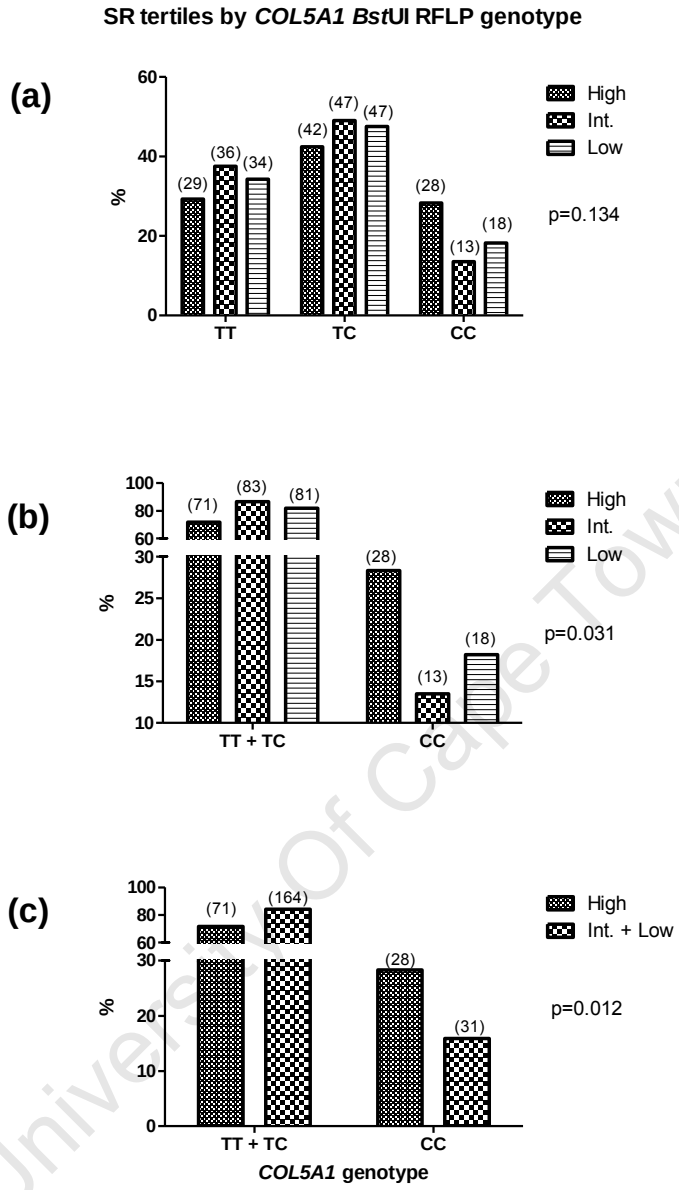

Figure 3.4. The *COL5A1 Bst*UI restriction fragment length polymorphism (RFLP) genotype distributions (% subjects) within the sit and reach (SR) High, Intermediate (Int.) and Low tertile groups, examined as **(a)** TT vs TC vs CC, **(b)** T allele vs CC, and **(c)** T allele vs CC, with Intermediate and Low tertiles combined for comparison against the High SR tertile. Genotype frequencies were significantly different when the T allele was compared to CC genotype for High vs Int. vs Low (p=0.031) in **(b)** and for High vs Int. + Low (p=0.014) in **(c)**.The number of samples (N) is indicated in parenthesis above each bar.

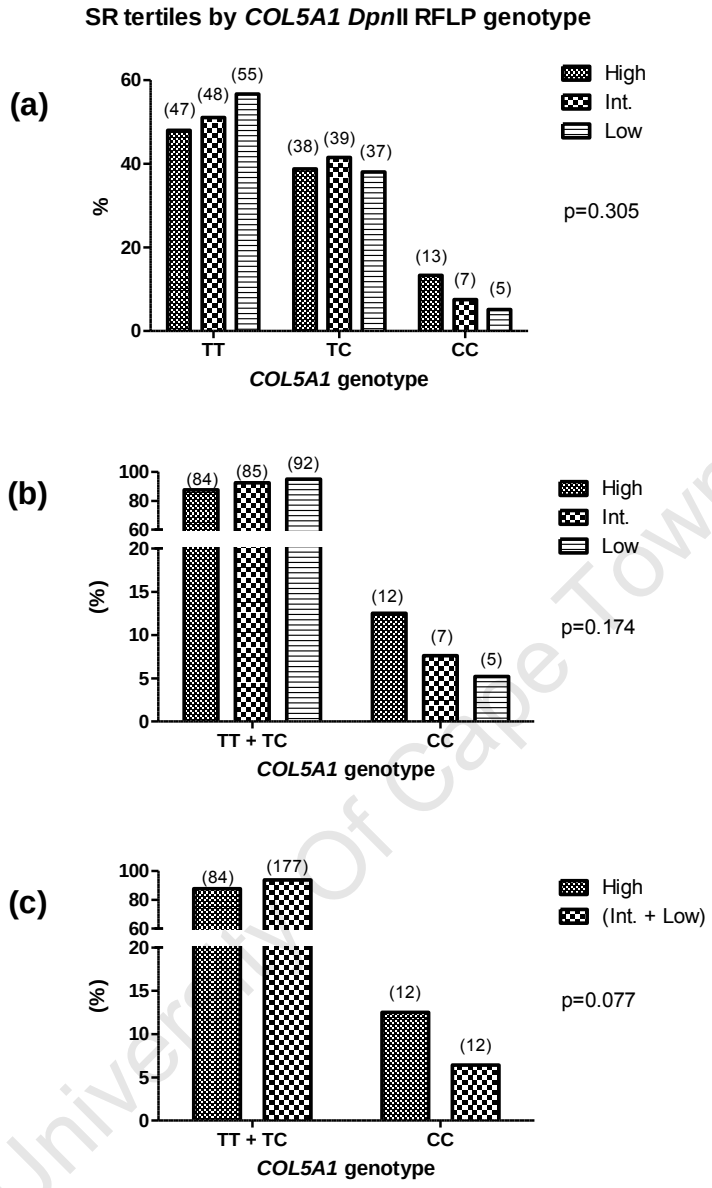

Figure 3.5. The *COL5A1 Dpn*II restriction fragment length polymorphism (RFLP) genotype distributions (% subjects) within the sit and reach (SR) High, Intermediate (Int.) and Low tertile groups, examined as **(a)** TT vs TC vs CC, **(b)** T allele vs CC, and **(c)** T allele vs CC, with Intermediate and Low tertiles combined for comparison against High SR tertile. There was a significant (p=0.044) linear trend when the T allele was compared against the CC genotype in **(b)**. The number of samples (N) is indicated in parenthesis above each bar.

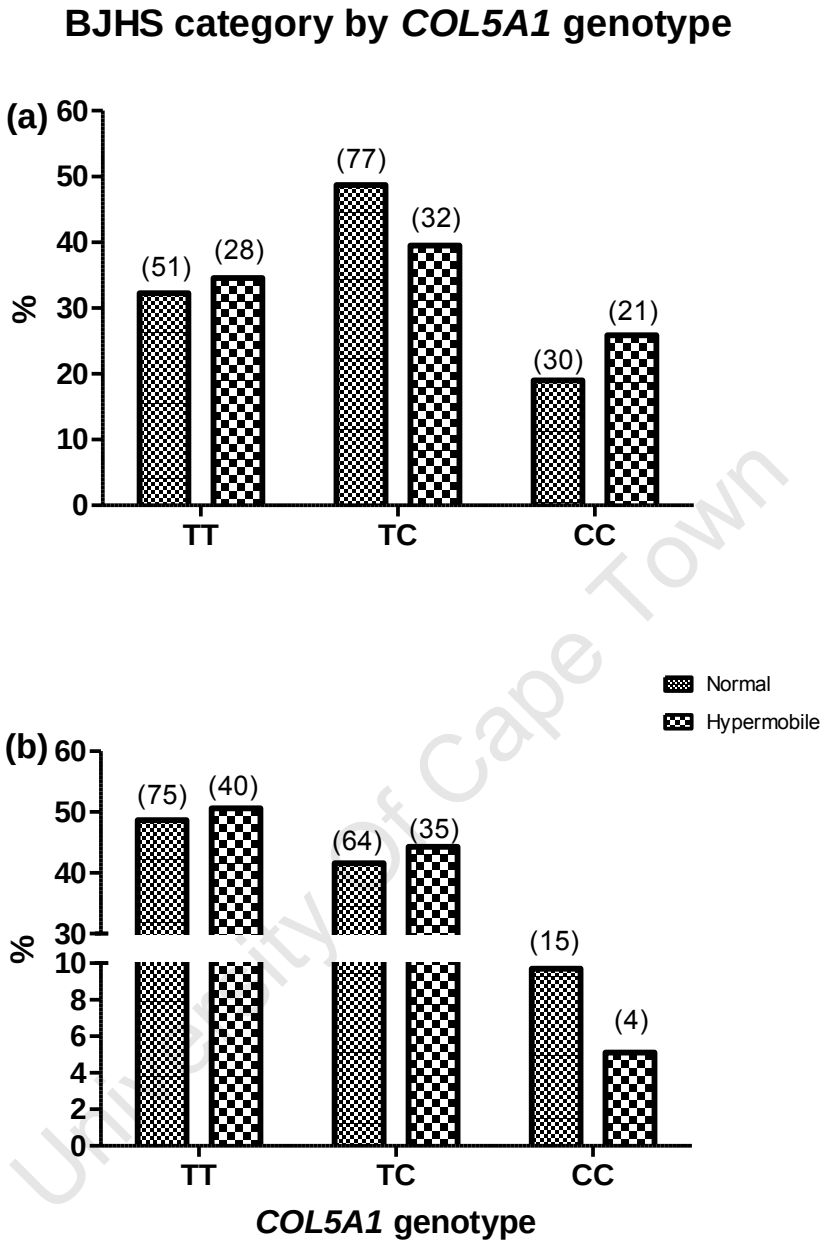

Figure 3.6. The *COL5A1* **(a)** *Bst*UI and **(b)** *Dpn*II restriction fragment length polymorphism (RFLP) genotype distributions (% subjects) within the normal and hypermobile subjects as determined by the benign joint hypermobility score (BJHS). The number of samples (N) is indicated in parenthesis above each bar

3.3.4. Correlations and interactions between sit and reach (SR) ROM measurements and non-genetic extrinsic and intrinsic factors in. the *COL5A1* genotypes

We used multiple regression analyses to determine whether genotype was interacting with any non-genetic factors commonly associated with ROM in the literature (Chapter 1, Section 1.6). A significant interaction (P=0.024) between age and the *COL5A1 Bst*UI genotypes was found for SR ROM. The rate of change in SR ROM with age was different between genotypes (Figure 3.7). While there was a tendency, although not significant, for SR ROM to decrease with increasing age for the TT (r=-0.15) and TC (r=-0.11) genotypes, there was a significant tendency for an increase in the relationship for the CC genotype (r=0.25). Owing to the similar relationship of SR ROM with increasing age of the *Bst*UI TT and TC genotype groups, these genotypes were grouped together for comparison against the *Bst*UI CC genotype in the final graph (Figure 3.7d). The interaction between SR ROM and age, when analysed for the T allele against the CC genotype, was very significant (p=0.007). While not a significant interaction between for the *Dpn*II RFLP genotypes (p=0.351), there was a similar response of SR ROM to increasing age for all three genotypes (Appendix G5, Figure G.3). Although not significant (p=0.114), we report an interesting observation with respect to the interaction between SR ROM and the amount of reported flexibility training between *COL5A1* BstUI RFLP genotypes (Figure 3.8). While the SR ROM of the *Bst*UI RFLP TT genotype (p=0.028) increased significantly with

increasing amount of reported flexibility training, the other two genotypes had no significant correlation between these two variables. No other interactions or correlations between SR ROM measures and intrinsic or extrinsic factors with *Bst*UI or *Dpn*II genotypes were found (Appendix H4, Tables H.1 and H.2).

Similarly, no interactions between the SLR or the ShTR ROM assessments and these factors were reported in either RFLP genotypes (data not shown).

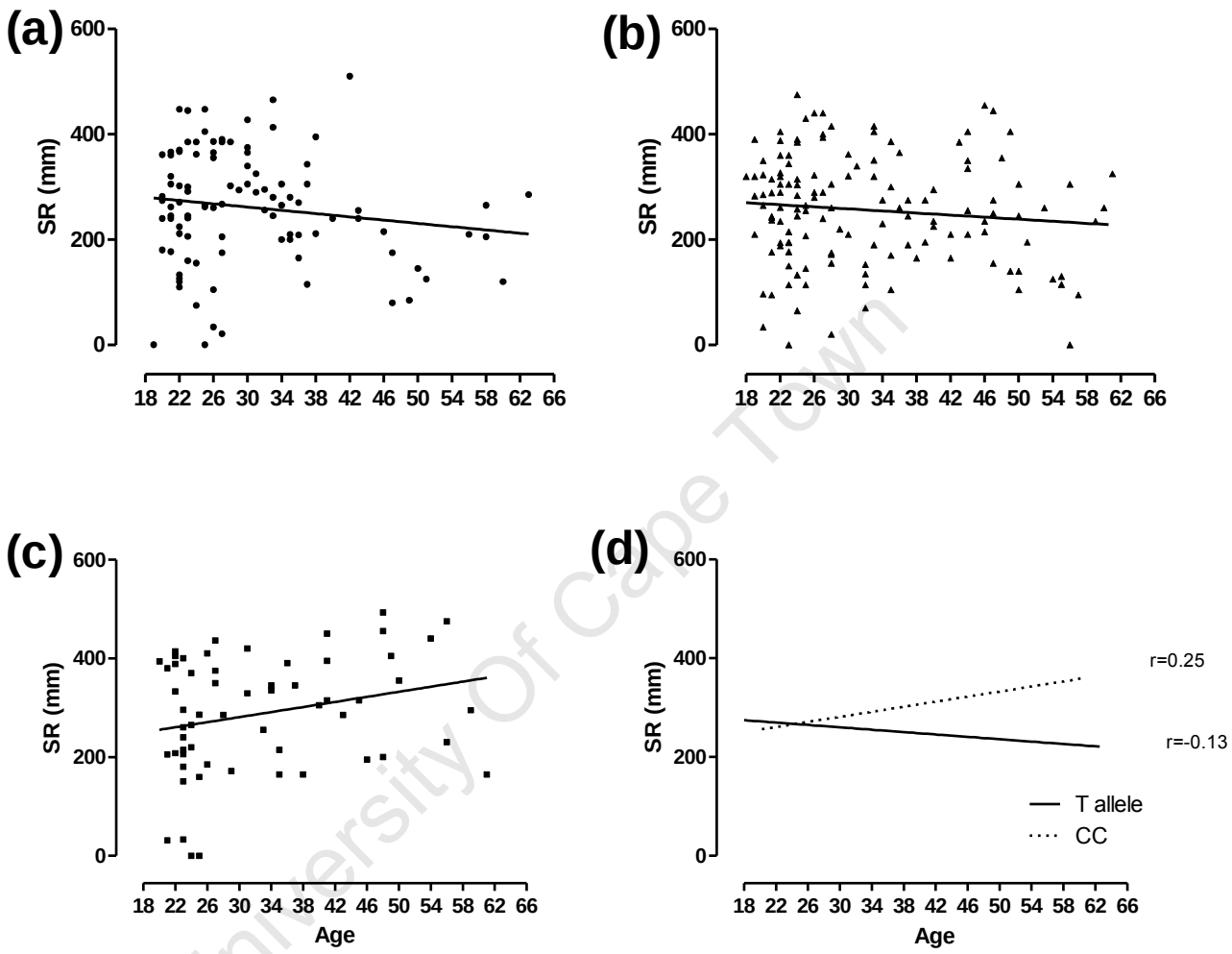

Figure 3.7. The relationship between sit and reach measurement with increasing age for each *COL5A1* BstUI RFLP genotype. **(a)** TT genotype, **(b)** TC genotype, **(c)** CC genotype and **(d)** T allele (TT and TC) vs CC genotype. r value is the correlation value the two factors (SR and age). Genotype correlations were represented individually to aid visualization. Owing to the similar relationship of the TT and TC genotype, these were combined for analysis against the CC genotype in **(d)**.

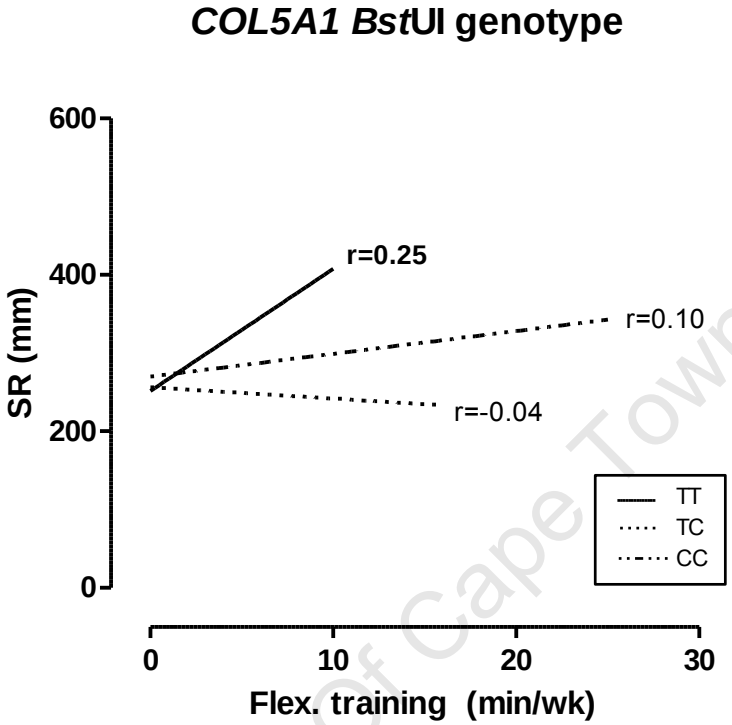

Figure 3.8. SR ROM by flexibility training for the *Bst*UI genotype. Values in bold emphasize a significant correlation.

SR - sit and reach; Flex. - Flexibility; min - minutes; wk - week

3.3.5. "Young" and "old" sub-samples and the differences in their association with *Bst*UI and *Dpn*II RFLP genotypes for ROM measurements.

Based on the discovery of the divergence of SR ROM with age in *Bst*UI genotypes (Figure 3.7), the sample was divided into two age groups for further analysis. Those younger than 35 years of age were classified as "young" and those 35 years or older were classified as "old". A cut-point of 35 yrs of age was chosen as this was the age at which there was sufficient divergence in SR ROM between genotypes, but an adequate sample in each group to facilitate subsequent comparisons. The general and ROM characteristics of the "young" and "old" age groups are reported in Table 3.5.

Table 3.5: General characteristics and range of motion (ROM) of the "young" (age<35 years) and "old" (age ≥ 35 years) age groups.

	Young	Old	p-value [a]
General characteristics			
Gender (% males)	61.8 (212)	65.1 (109)	0.557
Age (years)	25.2 ± 4.2 (212)	45.1 ± 7.8 (109)	**<0.001**[c]
Height (m)	**1.75 ± 0.09 (191)**	**1.75 ± 0.09 (61)**	0.632 [b]
Weight (kg)	73.2 ± 13.2 (191)	**72.6 ± 12.6 (61)**	0.472 [b]
BMI (kg/m^2)	23.8 ± 2.9 (190)	23.6 ± 2.7 (60)	0.553 [b]
Waist circumference (cm)	**77.8 ± 8.1 (142)**	78.6 ± 10.8 (13)	0.322 [b]
Flex training (min/wk)	**1.18 ± 2.6 (173)**	2.36 ± 5.5 (70)	**0.023**
Students (%)	59.0 (156)	1.6 (63)	**<0.001**
Current training (hr/week)	**8.6 ± 6.3 (120)**	8.2 ± 6.9 (15)	0.812
Hypermobile (%)	34.8 (181)	27.9 (68)	0.311
Current injury (% "Yes") [d]	19.4 (180)	38.0 (79)	**0.002**
CT injury/disorder (% "Yes") [e]	47.2 (180)	46.8 (79)	0.954
ROM measurements			
SR (mm)	267 ± 110 (204)	259 ± 105 (109)	0.598 [b]
Non-dom SLR (°)	86 ± 24 (41)	80 ± 23 (17)	0.132 [b]
Non-dom ShTR (°)	105 ± 17 (157)	100 ± 19 (20)	0.148 [b]

Values are expressed as average ± standard deviation or as a frequency. The number of subjects (N) is in parentheses.
[a] Young vs Old,
[b] P-value adjusted for gender
[c] Levene's test of homogeneity revealed this variable was not normally distributed and therefore results should be interpreted with caution
[d] non-serious (did not require hospitalization or immobilization).
[e] previous history of either a connective tissue injury or disorder
Dom – dominant; Flex – flexibility; min – minutes; wk – week; SLR - straight leg raise; ShTR - shoulder total rotation; CT - connective tissue.
Significant differences are in bold (p<0.05), while bold values in body of the table indicate significant correlations with SR scores for either age group.

All ROM measurements, and the majority of descriptive data were matched for both age groups, after adjusting for gender. Importantly, there was no difference in the amount of reported sport training ("current training") performed per week by the "young" and "old" sub-samples. As expected, age ($p<0.001$), the prevalence of students ($p<0.001$) and the reporting of a current, non-serious injury ($p=0.023$) were significantly different between the two groups. Although a significant difference for the Levene's test of homogeneity for age indicated that this statistic was not normally distributed, one would expect this variable to be significantly different between the groups. Approximately 59% of the "young" group reported being a student, while only 2% reported the same occupation in the "old" group. The discovery of a higher prevalence of non-serious injury in the "old" age group was expected from the previous finding in Section 2.3.1 of Chapter 2.

In contrast, the finding that the "old" group reported performing significantly more flexibility training than the "young" group (mean = 2.36 ± 5.5 min/wk vs. 1.18 ± 2.6 min/wk, $p=0.023$) was unexpected.

The "young" and "old" groups were also examined separately for correlations with these non-genetic intrinsic and extrinsic factors (the full data of these analyses in presented in Appendix H6, Table H.3). If a factor was significantly correlated with the SR measurements of either the "young" or "old" group, it was highlighted in Table 3.5. Height was significantly negatively correlated in both groups ("young": $r=-0.18$, $N=183$, $p=0.015$; "old": $r=-0.45$, $N=61$, $p<0.001$). Weight was only

significantly negatively correlated with SR ROM in the “old” age group (r=-0.43, N=61, p=0.001). In contrast, waist circumference (r=-0.19, N=137, p=0.023), the amount of reported flexibility training (r=0.16, N=166, p=0.041) and the reported current amount of training (r=-0.23, N=120, p=0.012) were significantly correlated in the “young” age group only.

The “young” and “old” groups were examined separately for *COL5A1 Bst*UI and *Dpn*II RFLP genotype-dependent differences in the ROM measurements (Table 3.6 and 3.7, respectively). Furthermore, based on the findings presented in Figure 3.7, the T allele of the *Bst*UI RFLP (TT and TC genotypes) was also compared to CC genotype. Owing to differences reported in Chapter 2 (Section 2.3), the SR ROM measurements were covaried for the effect of gender, weight and exercise in the 24 before hours prior to the first visit. With similar justification, the non-dominant SLR and non-dominant ShTR measurements were covaried for gender and weight.

Table 3.6. Comparison of the range of motion (ROM) measurements between *Bst*UI genotype groups within the "young" (<35 years) and "old" (≥35 years) age groups.

	"Young" Age Group (<35 years)				**"Old" Age Group** (≥35 years)			
	TT	**TC**	**CC**	**p-value** [a]	**TT**	**TC**	**CC**	**p-value** [a]
SR (mm)	274 ± 110 (73)	262 ± 106 (87)	269 ± 123 (37)	0.786 [c]	225 ± 96 (26)	245 ± 100 (48)	321 ± 108 (22)	**0.017** [b, c]
T allele vs CC	267 ± 108 (160)		269 ± 123 (37)	0.555 [c]	238 ± 98 (74)		321 ± 108 (22)	**0.004** [c]
Non-dom SLR (°)	77 ± 22 (12)	93 ± 24 (21)	83 ± 23 (8)	0.182 [d]	82 ± 23 (5)	77 ± 22 (10)	90 ± 41 (2)	0.776 [d]
T allele vs CC	87 ± 24 (33)		83 ± 23 (8)	0.410 [d]	79 ± 15 (15)		90 ± 41 (2)	0.468 [d]
Non-dom ShTR (°)	200 ± 28 (52)	208 ± 28 (75)	204 ± 31 (30)	0.355 [d]	204 ± 17 (7)	194 ± 37 (11)	204.0 ± 38.2 (2)	0.770 [d]
T allele vs CC	205 ± 28 (127)		204 ± 31 (30)	0.806 [d]	198 ± 30 (18)		204.0 ± 38.2 (2)	0.803 [d]

Values are expressed as average ± standard deviation with the number of subjects (N) in parentheses.
[a] TT vs TC vs CC or T allele (TT + TC) vs CC.
[b] Pair-wise post-hoc significant differences: TT vs CC, p=0.004, TC vs CC, p=0.006
[c] Value adjusted for gender, weight and exercise in the 24 hours before the first testing session.
[d] Value adjusted for gender and weight
Significant differences are in bold font.
T allele - TT genotype + TC genotype; Dom - dominant; SR - sit and reach; SLR - straight leg raise; ShTR - shoulder total rotation.

Table 3.7. Comparison of the range of motion (ROM) measurements between *Dpn*II genotype groups within the "young" (<35 years) and "old" (≥35 years) age groups.

	"Young" Age Group (<35 years)				**"Old" Age Group** (≥35 years)			
	TT	**TC**	**CC**	**p-value** [a]	**TT**	**TC**	**CC**	**p-value** [a]
SR (mm)	263 ± 114 (101)	272 ± 111 (75)	280 ± 108 (15)	0.796 [b]	241 ± 111 (46)	255 ± 91 (38)	332 ± 115 (10)	0.089 [b]
T allele vs CC	226 ± 113 (176)		280 ± 108 (15)	0.952 [b]	244 ± 98 (83)		332 ± 115 (10)	**0.032** [b]
Non-dom SLR (°)	82 ± 23 (19)	93 ± 22 (19)	68 ± 40 (2)	0.174 [c]	87 ± 23 (9)	72 ± 9 (5)	90 ± 41 (2)	0.442 [c]
T allele vs CC	87 ± 23 (39)		68 ± 40 (2)	0.621 [c]	78 ± 18 (13)		90 ± 41 (2)	0.759 [c]
Non dom ShTR (°)	203 ± 28 (84)	209 ± 28 (64)	190 ± 33 (9)	0.115 [c]	200 ± 22 (11)	193 ± 46 (6)	204 ± 38 (2)	0.866 [c]
T allele vs CC	206 ± 28 (148)		190 ± 33 (9)	0.758 [c]	196 ± 31 (16)		204 ± 38 (2)	0.548 [c]

Values are expressed as average ± standard deviation with the number of subjects (N) in parentheses.
[a] TT vs TC vs CC or T allele (TT + TC) vs CC.
[b] Value adjusted for gender, weight and exercise in the 24 hours before the first testing session.
[c] Value adjusted for gender and weight
Significant differences are in bold font.
T allele - TT genotype + TC genotype; Dom - dominant; SR - sit and reach; SLR - straight leg raise; ShTR - shoulder total rotation.

There were no significant differences in ROM measurements between the genotype groups of the *COL5A1 Bst*UI or *Dpn*II RFLPs of the "young" sample, even after adjusting for confounding factors (Tables 3.6 and 3.7). This finding was consistent whether the sample was divided into the three genotype groups of each variant or when the T allele of each variant was compared to the respective CC genotype. However, the mean SR ROM values were significantly different between the three *COL5A1 Bst*UI RFLP genotype groups in the "old" sample (TT=225 ± 96 mm, TC=245 ± 100 mm, CC=321 ± 108mm, p=0.017) (Table 3.6). When combined, the average SR measurement of *Bst*UI RFLP T allele group was significantly lower than the *Bst*UI RFLP CC genotype group (238 ± 98 mm vs 321 ± 108 mm, p=0.004). Similarly, in the "old" age group, the average SR measurement of the *Dpn*II RFLP T allele was significantly lower than the CC genotype group (244 ± 98 mm vs 332 ± 115mm, p=0.032) (Table 3.7).

Furthermore, *COL5A1 Bst*UI and *Dpn*II genotype distributions within SR ROM tertiles were re-examined in these age categories of "young" and "old" (Figure 3.9). Consistent with the findings presented in Tables 3.6 and 3.7, there were no significant differences in the genotype distributions within the SR tertiles for the "young" group for either RFLP. There was, however, a significant over-representation of the CC genotype content of the High SR ROM tertiles of the "old" age group for both the *Bst*UI (p=0.004) and *Dpn*II (p=0.009) RFLPs. Note that, due to small sample sizes, the Intermediate and Low SR ROM tertiles were combined for the *Dpn*II RFLP.

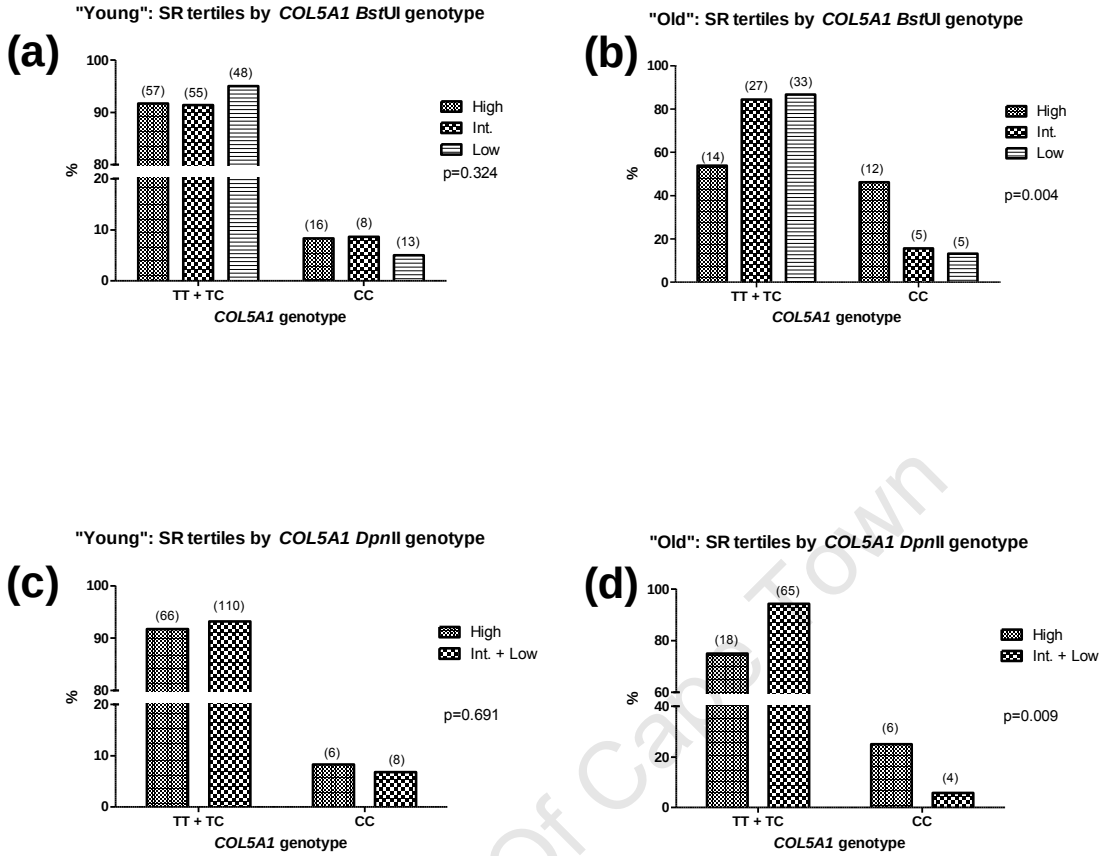

Figure 3.9. "Young" and "Old" *COL5A1 Bst*UI and *Dpn*II restriction fragment length polymorphism (RFLP) genotype distributions (% subjects) within the sit and reach (SR) High, Intermediate (Int.) and Low tertile groups. The T allele (TT + TC) was compared to CC genotype for **(a)** "Young" *Bst*UI RFLP, (**b)** "Old" *Bst*UI RFLP, **(c)** "Young" *Dpn*II T allele, and **(d)** "Old" *Dpn*II RFLP. For the DpnII RFLP, the Intermediate and Low tertiles were combined for comparison against the High SR tertile due to low sample sizes. The genotype distribution is significantly different for "Old" group of both the *Bst*UI (p=0.004) and *Dpn*II (p=0.009) RFLPs.

3.3.6. Determinants of SR ROM in the "Old" group.

Multivariate analysis was used to describe the relationships between intrinsic or extrinsic factors, as well as *COL5A1 Bst*UI and *Dpn*II RFLP genotypes, on SR ROM in the "old" group (Table 3.8). Factors that were significantly associated with SR ROM in this cohort (Chapters 2 and 3) were entered into the model. Forward stepwise regression analysis was performed on the following factors: gender, weight, exercise in the 24 hours prior to the first visit, primary sport's association with lower body ROM and *Bst*UI or *Dpn*II genotype (TT and TC combined for comparison against CC). When *Bst*UI RFLP genotype was included in the model, only gender ($p<0.001$) and *Bst*UI genotype ($p<0.001$) were significant determinants and explained 22.8% of the variance in SR ROM ($p<0.00001$). Similarly, when *Dpn*II RFLP genotype was included in the model, only gender ($p=0.001$) and *Dpn*II genotype ($p=0.020$) contributed significantly to the model and explained 17.3% of the variance in SR ROM ($p<0.00020$). Weight, exercise in the 24 hours prior to the first visit and primary sport's association with lower body ROM were not significant determinants in these models.

Table 3.8: Multivariate analysis for the SR ROM in the "old" age group (≥ 35 years) including the *Bst*UI and *Dpn*II RFLP genotypes, separately.

	β	B	p-value
For the overall SR ROM for the BstUI RFLP, R = 0.478, R^2 = 0.228, SEE = 94.1, p<0.00001.			
***Bst*UI RFLP**			
Gender	-0.350	-77.18	**<0.001**
Genotype (TT+ TC vs CC)	0.288	72.31	**<0.001**
For overall SR ROM DpnII RFLP, R = 0.415, R^2 = 0.173, SEE = 94.9, p<0.00020.			
***Dpn*II RFLP**			
Gender	-0.322	-69.42	**0.001**
Genotype (TT+ TC vs CC)	0.229	75.83	**0.020**

SEE - Standard Error of estimate; β - partial correlation coefficient; B - parameter estimate.

3.4 Discussion

The main, and novel finding, of this study was that SR ROM was significantly associated with *Bst*UI and *Dpn*II RFLP genotypes of the *COL5A1* gene in an "old", but not a "young", apparently healthy and physically active population. Specifically, there was a significant interaction between the *COL5A1 Bst*UI genotypes and age with SR ROM, such that SR ROM increased with age in those with the CC genotype, while there was little or no correlation between SR ROM and age in those with the T allele (TT and TC genotype). While this interaction effect was not significant for the *Dpn*II RFLP, the genotypes exhibited a similar pattern of divergence with increasing age, resulting in the CC genotype

of both these RFLPs being associated with increased SR ROM in comparison to those with a T allele (TT + TC genotypes) in the older subjects.

The finding that ROM is associated with *COL5A1* sequence variants in this dissertation supports, to a certain extent, previously published work from this laboratory [97]. In a mixed cohort, consisting of subjects with a history of chronic Achilles tendinopathy and Achilles tendon rupture, as well as, those with no history of a tendon injury, the *COL5A1 Bst*UI RFLP was associated with SR and SLR ROM [97]. While the present study concluded that TT and TC genotypes had less ROM than the CC genotype, the previously published work [97] concluded that the TC genotype had significantly less ROM than the TT and CC genotypes. While the rate of change of SR ROM with increasing age was very similar for all three genotypes in the previous study, a similar divergence to that observed among our cohort's genotypes with increasing age, emerged once the uninjured controls were analysed separately. Furthermore, this divergence was absent when the injured group were analysed separately [113]. However, due to small sample sizes in these two groups in the previous work, this preliminary finding requires further research. Subjects who had suffered a serious injury (incident to musculotendinous tissue that required hospitalization or immobilization) in the 24 months before testing were excluded from our cohort. This was confirmed through two methods – (1), a pre-testing interview and, (2) a self-reported detailed questionnaire.

The association of ROM with age has been detailed previously in Chapter 1 (section 1.6.2.1). The theory that this reduction in ROM is a result of a decline in physical activity [64] is not tenable in our particular cohort. The "old" sub-sample reported very similar amounts of training per week to the "young" sub-sample (Table 3.5). Furthermore, there were no differences in ROM values between the two age categories. Despite this homogeneity between age groups, there was a distinct difference in the SR ROM response to aging, when the cohort was examined by *COL5A1 Bst*UI and *Dpn*II RFLP genotype. The T allele exhibited a non-significant decline in SR ROM in both *COL5A1* RFLPs with increasing age. This decline is expected from reports in the literature (Chapter 1, Section 1.6.2.1), and could be explained by the biochemical-mediated theory proposed in the ACSM position stand [69]. In brief, this theory attributes the decline in ROM to an increase in tendon rigidity with increasing age. In contrast to those possessing a T allele, the "CC" genotype would appear to be "protected" from this biochemical-mediated decline in SR ROM in both RFLPs.

Within the "old group", gender and *COL5A1 Bst*UI or *Dpn*II genotype, explained a significant percentage of SR ROM variance in our cohort (Table 3.8) (*Bst*UI RFLP - 22.8%, $p<0.00001$; DpnII RFLP - 17.3%, $p<0.00020$). While gender has frequently been reported as an important contributing factor to variance of ROM previously (Chapter 1, Section 1.6.2.2), genotype has seldom been considered in ROM studies of apparently healthy cohorts. Furthermore, the significant association of reported flexibility training with the *Bst*UI TT, but not other

genotypes suggests a genotype association in the response to stretching. Both these results presented in this study suggest that genotype-phenotype interaction in an important trait such as ROM should always be considered in future studies.

Interestingly, there were significant differences in the two anthropometric variables - weight and waist circumference in both the investigated RFLPs. (Tables 3.1 and 3.2). However, in contrast to the rate of change of SR ROM with aging, weight and waist circumference were consistently negatively correlated with SR ROM for all genotypes. The finding can not be fully explained, but is worth exploring in future studies as it suggests a genotype association with weight in an apparently healthy and physically active population. Furthermore, this finding should encourage waist circumference to be considered in future research on ROM, as it has not been reported commonly as a contributing intrinsic factor.

In contrast to Chapter 2, the discovery of correlations between commonly reported intrinsic and extrinsic factors and ROM became evident only once the cohort was examined by genotype. This is important contrast as it emphasises the importance of considering genotype-phenotype interaction for common traits, such as ROM.

Of note, were two findings when the cohort was divided into tertiles based on SR ROM. Firstly, the intermediate, rather than the high, ROM group reported the

greatest amount of flexibility training (Table 3.4). Secondly, the reporting of a non-serious current injury was significantly higher in the intermediate than the high or low ROM tertiles. This is in contrast to reports in the literature (Chapter 1, Section 1.2) which regard a high or low ROM as the highest risk factor for musculoskeletal injury. These findings could be explained by the fact that common rehabilitation for prior injuries is flexibility training (stretching) to return ROM to pre-injury levels [37]. However, this is an issue that a cross-sectional study is incapable of addressing and this is only a theory proposed by the author to explain these two deviations from the norm.

The main limitations of this study were the small sample size for the "old" group in comparison to the "young" group, particularly when analysing the cohort by genotype. When comparing the amount of training in "old" and "young" sub-samples, there were far less reported information for the "old" group. This is due to the fact that the majority of the "old" group had been recruited from running events, which used a different version of the questionnaire. However, the fact that these subjects were recruited from marathon and ultra-marathon running events would imply that these individuals were also physically active.

Furthermore, the low sample size for the SLR assessment meant that the SR ROM findings could not be confirmed with this highly repeatable lower body assessment. While sample sizes were larger for the shoulder ROM, and despite using a clinically valid assessment [19], the reliability of these assessments were

too low to detect small differences in genotype groups. This limitation is discussed in greater detail in Chapter 4, Section 4.3 Similarly, the low sample size for waist circumference measures meant that this variable could not be adequately investigated for genotype associations, which were suggested by the differences in weight between genotypes. The fact that the LOA for the SR Bland-Altman analysis (Table 2.1 and Appendix F1) suggests that the differences in SR means should be interpreted with caution to prevent a Type I error being committed by the author. However, the fact that the distribution of T allele and CC genotype groups among SR ROM tertiles was also significantly different for both the *Bst*UI and *Dpn*II RFLPs (Figures 3.4 and 3.5, respectively), confirms that there is indeed an association with of SR ROM with genotype. Furthermore, it is the genotype interaction with SR ROM with age, rather than the exact measure of difference in SR ROM means between genotype groups, that is the most important finding of this dissertation. Another limitation of the study was the uncontrolled measure of flexibility training. This was only gained from a self-reported questionnaire and this factor could only properly be investigated by designing a randomized control trial to assess the effect of a regulated intervention of flexibility training on ROM.

In conclusion, the *Bst*UI and *Dpn*II RFLP genotypes were associated with SR ROM measures in an apparently healthy and physically active cohort. Notably, this association becomes more apparent with age due to the interaction of age with genotype, significantly with *Bst*UI RFLP, and with a similar non-significant

trend for the *Dpn*II RFLP. While the SR ROM of the T alleles of both RFLPs declined with age (as is expected), the CC genotype was “protected” against this decline. Furthermore, there was a significant association with the amount of reported flexibility training with one (TT) of the *Bst*UI genotypes, but not the others. This chapter has emphasized the importance of considering genotype, in conjunction with other commonly reported phenotype factors, as an important contributor to the observed SR ROM variance in an apparently healthy and physically active cohort.

Chapter 4

Intrinsic and extrinsic factors associated with ROM in an apparently healthy and physically active population: Summary of results, clinical relevance and future directions

4.1 Summary of results

In this dissertation, common extrinsic and intrinsic factors that have been associated with ROM previously, as well as a novel genetic factor (variants within the *COL5A1* gene), were investigated in an apparently healthy and physically active cohort.

The main findings of this dissertation were that only three of the eight non-genetic factors (Table 4.1) commonly associated with ROM were independently associated with SR ROM in this cohort – (1) level and type of sport participation, (2) gender and (3) limb dominance. Those sports that had been associated with reduced ROM in the literature and by subjective comparison (Appendix F, Table F.3, with references included in this table) were associated with reduced SR ROM in this cohort. In contrast, those sports that have previously been associated with increased ROM, by the same method of assessment, were associated with greater SR ROM in this cohort. As expected from the review of the literature (Section 1.6), females were associated with significantly greater

ROM in almost all ROM assessments (except for the non-dominant shoulder internal rotation) in comparison to males. The dominant limb was associated with greater ROM for the SLR and ShER, while the non-dominant limb was associated with greater ROM for the ShIR in comparison to the contralateral limb. As reviewed in chapter 1 and summarized in table 4.1 all three of these factors are all associated with ROM with a high level of certainty and would therefore be expected to be associated with ROM in this cohort.

In contrast, the lack of association with two other high certainty factors - age and flexibility training - was unexpected and could not initially be explained by the results presented in Chapter 2. The fact that the majority of the subjects in this cohort (34%, N=128) were younger than 25 years of age could not be excluded as a possible explanation for the lack of association of age with ROM. However, the investigation of the association of the *COL5A1* genotypes (Chapter 3) in conjunction with these, and other phenotypic factors commonly associated with ROM, explained some of the lack of associations in the initial results chapter (Chapter 2). The most important novel finding of this dissertation was the significant interaction between *COL5A1 Bst*UI RFLP genotypes and age for SR ROM. Although not significant, there was a similar interaction trend for the *Dpn*II RFLP genotypes.

Table 4.1. Common and novel intrinsic and extrinsic factors associated with ROM, and the level of certainty of this association (Section 1.6), that were investigated in the dissertation. Whether the factor displayed any associations with ROM in the cohort is noted.

Intrinsic/ extrinsic	Factor	Certainty	Association or interaction in cohort
Extrinsic	Level and type of activity	High	**Reduced SR ROM in those that reported a sport associated with reduced ROM**
Intrinsic	Age	High	**Interaction with *COL5A1 Bst*UI RFLP genotype for SR ROM. CC genotype "protected" against age-related decline in ROM**
	Gender	High	**Females associated with increased SR, as well as non-dom. SLR and ShTR, ROM.**
	Limb dominance	High	**Dom. SLR and ShER, as well as non-dom. ShIR, associated with greater ROM in comparison to contralateral limb**
	Flexibility training	High	***Bst*UI RFLP TT allele significantly associated with greater ROM**
	Prior injury	Moderate	No association
	Weight/BMI	Moderate	No association
	Height	Low	No association
	Genotype	Low	**Both RFLPs associated with SR ROM in "old" (≥35 years old) group.**

Dom. - dominant; SR - sit and reach; SLR - straight leg raise; Sh - shoulder; IR - internal rotation; ER - external rotation; ROM - range of motion.

Specifically, the CC genotype was “protected” against the expected age-related decline in ROM (Section 1.6.2.1). This divergence in association of SR ROM with age was evident among the *COL5A1 Bst*UI and *Dpn*II RFLP genotypes from about from about 30-35 years of age onwards (this interaction was significant in the *Bst*UI, but not the *Dpn*II RFLP). Although not significant, a similar pattern of divergence was evident when the uninjured controls of previous work in this department were analysed [113]. Furthermore the “protective” effect of the CC genotype against the age-related decline in ROM was absent in the injured group of this cohort [113]. When, examined by “young” and “old” age groups in our apparently healthy and physically active cohort, this divergence phenomenon resulted in significantly different SR ROM means for the *Bst*UI and *Dpn*II genotypes in the “old” group. For the *Bst*UI RFLP, this difference was only significant if the TT and TC genotype groups were combined and compared against the CC genotype SR ROM.

It is of interest to note that the CC genotype of the *COL5A1 Bst*UI RFLP is also associated with decreased risk of developing Achilles tendinopathy [114] and acute ACL ruptures, in females [115]. Preliminary data from our laboratory suggests that the *Bst*UI RFLP C allele is associated with decreased *COL5A1* mRNA stability (M-J. Laguette, unpublished data). As previously discussed (Section 1.6.2.10), the *COL5A1* gene encodes for the alpha 1 chain of type V collagen. Type V collagen interacts with most abundant collagen, Type I collagen, and forms a template on which fibrillogenesis can occur. Importantly, type V collagen is also

thought to have a regulatory or "modulating" function of this fibrillogenesis [95]. The preliminary finding that *Bst*UI RFLP sequence variants are associated with *COL5A1* mRNA stability would suggest that these variants are associated with type V collagen levels. If confirmed, this hypothesis could provide an explanation for the observed variation in ROM among different genotypes in the "old" group of our cohort. However, exactly how type V collagen production, and therefore the diameter of collagen fibrils, is related to variation in ROM is not known. Further molecular research is required to investigate the possible mechanism of a relationship between type V collagen levels and protection against the age-related decline in ROM and/or musculoskeletal soft-tissue injuries.

Mutations within the *COL5A1* gene have also been shown to cause Ehlers-Danlos Syndrome (EDS), a connective tissue disorder characterized by, amongst other clinical signs, joint hypermobility [1]. It is interesting to speculate that sequence variants (disease causing mutations or polymorphisms) within the *COL5A1* gene, as well as other extracellular matrix encoding genes, are associated or cause the large variation in both pathological and normal ROM (refer to figure 1.3). Mutations within, amongst other extracellular matrix encoding genes, *COL1A1*, *COL1A2*, *COL5A2* and *Tenascin-X* cause EDS and other heritable disorder of connective tissue, which are characterized by joint hypermobility [32]. Further work is required to investigate the possible role of these genes, and their variants, in ROM.

Although not a significant interaction with either genotype, the amount of reported flexibility training (regular stretching) was positively associated with the TT, but not any other *COL5A1 Bst*UI genotypes, and SR ROM. Flexibility training or stretching is commonly suggested as an intervention to increase ROM in the general population [69]. Although the quantification of flexibility training could have been better controlled in this thesis, this preliminary finding suggests that the response of ROM to stretching could also be genotype-specific.

Furthermore, correlations with two other phenotype factors, commonly associated with ROM with a moderate to low level of certainty (Table 4.1), emerged only once the genotype of the cohort was considered. As expected from the literature reviewed in Chapter 1 (Section 1.6.2), weight was negatively correlated with SR ROM for the *Dpn*II TC genotype. Similarly, height was negatively correlated with the *Bst*UI TC genotype and the *Dpn*II TT and TC genotypes.

Notably, an intrinsic factor that has previously been shown to be associated with ROM with a moderate level of certainty (Section 1.6), prior injury, did not exhibit any association with SR ROM in the cohort investigated in this dissertation. However, this was not an unexpected finding due to the strict injury exclusion criteria of the study which required subjects to be free of serious (incident to musculotendinous tissue that required hospitalization or immobilization) injury in the 24 months before testing.

4.2 Clinical relevance

The ROM phenotype of an individual, as assessed by a clinician, is determined by a multitude of factors. ROM is a joint-specific trait and thus only a thorough examination can truly assess an individual's risk of injury [64]. Both high and low levels of flexibility are associated with an elevated risk of musculoskeletal injury (Section 1.5). To reduce the prevalence of injury an individual in a high injury risk area (Chapter 1, Figure 1.3) would need to be identified **prior** to an inciting event capable of resulting in an acute injury [3;8;107;116]. This would occur at a pre-season screening (athlete) or a general medical assessment (non-elite athlete) [108].

Practically, non-modifiable risk factors such as age, gender, limb dominance, prior injury, height and ethnicity can only provide the clinician with a comprehension for the divergence from the norm. On the other hand, modifiable factors such as level and type of sport participation, temperature, weight/BMI and flexibility training provide an opportunity for the clinician to introduce a lifestyle change or intervention to reduce the individual's risk of injury. Traditionally, flexibility training (regular stretching) has been advised for those with a reduced ROM [69 87 65], while one author has suggested that hypermobile joints should be strengthened and toned [11] to reduce the risk of injury.

However, this dissertation has highlighted the importance of considering a "personalised medicine approach" [108] when conducting a comprehensive

assessment and providing a suitable preventative intervention. For example, an apparently healthy and physically active individual with a *COL5A1 Bst*UI CC genotype would be expected to be protected from the age-related decline in ROM. Thus, if a reduced ROM is assessed in an older, apparently healthy and physically active individual with a CC genotype, factors other than advanced age should be considered to explain the deficit. Also, from preliminary findings in this thesis, an individual with a *COL5A1 Bst*UI TT genotype is suggested to be more responsive to flexibility training than those with a CC or TC genotype. Furthermore, the fact that two correlations with commonly associated ROM factors (weight/BMI and height) only emerged in our cohort once genotype was considered, also emphasises the importance of this personalised medicine approach to preventative medicine [107]. This type of medicine, considering individual genetic profiles to modify risk, has already been adopted by a high level Australian League rugby team [117].

4.3 Future studies in this field

The findings of this dissertation, in light of the previous work of Collins [97] and Posthumus [96], provides a greater understanding of the genetic association with *COL5A1* sequence variants and SR ROM and reiterates the importance of an personalised approach to medicine. However, rather than completing our understanding of the association of ROM with genotype, the findings of this

thesis has encouraged future research in this area with many unanswered questions.

A limitation of this current study was that the SLR was only measured in a sub-sample of the subjects and that the shoulder assessments were not reliable. However, both of these limitations have been discussed previously in Chapter 3 (Section 3.4). Although, as expected [104], there was a strong correlation between the SLR and SR measurements in our cohort (Appendix F6), the *COL5A1* genotype effects on SLR were not as consistent as those reported for the SR. Furthermore, despite performing the most clinically reliable shoulder assessments [18;19] on a larger cohort than the SLR, the large variability of the shoulder ROM measurements in this thesis (Section 2.28, Table 2.1) made the investigation of genotype association with this joint ROM difficult. Therefore, future studies should be performed on a large cohort with reliable upper and lower body ROM measurements to assess whether the genotype associations reported in Chapter 3 of this dissertation are general (systemic) or joint-specific and limited to SR ROM. One would expect there to be a fairly general (systemic) genotype effect on ROM measurements, but one should also not ignore the joint-specific influences of certain non-genetic factors, such as sports participation (Section 1.6.1.1). Also, the tentative association of flexibility training with genotype could be investigated further with a more controlled measure of the effectiveness and regularity of this reported stretching.

Furthermore, a wider range of single nucleotide polymorphisms (SNPs) could be investigated for associations with ROM. As previously mentioned (Section 4.1), other extracellular matrix encoding-genes that have been previously implicated in symptomatic joint hypermobility syndromes (e.g. EDS, Marfan Syndrome, OI, etc) are ideal candidate genes for this area of research [1]. Malfait [30] suggested a host of collagen-encoding genes - *COL1A1*, *COL1A2*, *COL3A1*, *COL5A1* and *COL5A2* - as well as a tenascin-encoding gene - *Tenascin-X* as being implicated in joint hypermobility. Grahame [32] listed a collagen-encoding gene - *COL3A1* - as well as two fibrillin-encoding genes - *FIB 1* and *FIB 2* as candidate genes for joint hypermobility. Also, the Trp2 allele of *COL9A2* has been associated with more "flexible" individuals in a cohort with sciatica [118]. Furthermore, the Cartilage Oligomeric Matrix Protein (COMP) gene has been suggested for genetic variant investigation due to reports of altered serum COMP levels in a cohort with Joint Hypermobility Syndrome [119]. With the increasing popularity and ease with which Genome Wide Association Studies (GWAS) can now be performed [120] there is no excuse for ignoring this important intrinsic factor of ROM.

Similarly, this investigation into the genotype association with ROM measurements should be expanded by investigating other ethnicities - a factor that is lacking good quality research (Section 1.6.2). Very large cohort studies could be less exclusive as far as this factor is concerned, as long as ethnic groups are analysed separately for to prevent any confounding effects of population stratification [109]. Similarly, the age range should be more evenly

distributed in a larger cohort. While our cohort had a broad age range, there was an uneven distribution of ages with the majority (69.2%) of subjects being younger than 35 years of age.

In conclusion, this dissertation has found an association between *Bst*UI and *Dpn*II *COL5A1* genotype and SR ROM in an apparently healthy and physically active population. A significant (*Bst*UI RFLP) and non-significant (*Dpn*II RFLP) interaction between genotype and age explained this observed difference in SR ROM in the "old", but not the "young" age group. Both reduced ROM and hypermobile individuals are at elevated risk of a musculotendinous injury [35]. Until now, only the phenotype of an individual has been considered to comprehend a deficit or excess of ROM. The findings of this dissertation suggest that a ROM assessment is incomplete without considering the genetic profile, at least of these two *COL5A1* sequence variants, of the individual. Furthermore, the genetic profile of an individual should also be considered when recommending a suitable intervention to increase ROM. While this study in isolation cannot provide all the answers for the genotype associations with ROM, and the modification thereof, it has highlighted the importance of research at the basic molecular level. Future, large genetic cohort studies, focused at the molecular level, and with reliable ROM assessments will be able to investigate some of the hypotheses proposed in the final Chapter of this dissertation.

Appendices

Appendix A – Summary of extrinsic (Table A1) and intrinsic (Table A2) factors associated with ROM as well as level of certainty [44] of association.

Table A.1 Summary of extrinsic factors associated with ROM, as well as a level certainty of association [44].

Factor investigated	Authors, journal and year of publication	Type of study and (level of evidence) [43]	Population studied	Joint/structure	Method of investigation	Conclusion	Assessment of study [44]	Overall level of certainty for factor [44]
Level and type of activity performed	Ekstrand, 1982, AJSM [13]	Retrospective (III)	Senior Division IV soccer players (M)	Hip, lower back, knee and ankle	Hip flexion, hip extension, hip abduction, knee flexion and ankle dorsiflexion (all performed with flexometer except hip abduction – goniometer)	Soccer players sig. less ROM in all measures except hip flexion in which soccer players sig. more ROM than reference group.	C	High
	Wang, 1993, JOSPT [27]	Case-control (III)	Long-distance runners and non-running controls (M + F)	Hamstrings, quadriceps and posterior calf muscles.	Modified straight leg raise, modified Thomas test, ankle dorsiflexion	Runners have significantly ($p<0.05$) reduced hamstring ROM in comparison to non-runners	B	
	Steinberg et al., 2006, AJSM [52]	Case-control (III)	Dancers and non-dancers (F)	Hip, knee, ankle and foot.	Ankle plantar flexion and dorsiflexion; hip rotation, abduction, extension; hip and knee flexion. Using goniometer, without	In 6 of the 7 ROM measures, dancers were sig. ($p<0.05$) more ROM than non-dancers. One measure showed no sig. differences.	C	

					warm-up.			
	Mafulli, 1994 BJSM [53]	Observational (III)	Elite young athletes, aged 9-18 years old practicing football, gymnastics, swimming and tennis (M + F)	Shoulder, trunk, “front split”, quadriceps.	Validated linear measures of muscle stiffness. Better of two attempts recorded. Following 2min warm-up.	Gymnasts most flexible in shoulder, hamstring/lumbar spine and hip joint ROM ($p<0.05$) measures.	C	
	Jones, 2002, Int. J Sports Med [61]	Observational (III)	International-standard long distance runners	Hamstring/hip Range of Motion (ROM).	SR test (standard), after 15 min self-paced running. Best of 5-6 attempts recorded.	Hamstring ROM sig. ($p<0.0001$) negatively correlated to running economy at 16km/h.	C	
	Ekstrand and Gillquist, Int. J Sports Med [54]	Prospective (I)	Senior soccer players (M)	Hip, knee and ankle ROM.	Hip Flexion, extension and abduction. Knee flexion and ankle dorsiflexion (hip abduction measured with goniometer, others	Soccer players sig. less ROM ($p<0.001$) than reference group in all measures except hip flexion.	B	

					measured with flexometer).			
	Kibler et al., 1996, AJSM [55]	Cross-sectional (III)	Elite tennis players, 14-21 years (M + F)	Shoulder ROM.	Shoulder internal and external rotation, measured with goniometer.	Internal and total shoulder ROM decreases sig. with playing years.	B	
	Huang et al., 2008, Int. J Sports Med. [29]	Cross-sectional (III)	Aikido athletes, freshman year (M + F)	Upper body ROM.	Goniometric protocols as described by Norkin and White. Very high ICCs for all measures.	Aikido athletes sig. more ROM than upper body and lower-body athletes in most measures.	B	
	Borsa et al., 2006, MSSE [58]	Cross-sectional (III)	Professional baseball players (M)	Shoulder ROM.	Supine forward elevation, internal and external rotation and horizontal adduction using goniometer. ICC values range widely	Throwing arm of professional baseball players have sig. more external ROM and sig. less internal ROM than non-throwing arm.	C	
Temperature	Mutungi and Ranatunga, 1998, J Physiol [63]	Cross-sectional (III)	Rat muscle	Viscoelasticity of muscle		Tension of muscle decreases with increase in temperature from 10 to	C	LOW

							35°C.	
BJSM – British Journal of Sports Medicine; SJSMM – Scandinavian Journal		Sawyer et al., 2003, J Strength Cond. Res.	Randomized Control Trial (RCT) (II)	Male subjects	Hamstring flexibility	Active Knee Extension (AKE)	Increasing hamstring temperature by 0.4°C had no effect on hamstring ROM.	B

of Medicine and Science in Sport; AJSM – American Journal of Sports Medicine; MSSE – Medicine and Science in Sports and Exercise.
SR - sit and reach; SLR – Straight Leg Raise; A.K.E – active knee extension; RCT – Randomized Control Trial; ROM – range of motion.
Adapted magnitude of net benefit: [44] for cross-sectional studies: A - Well designed RCT that found an association, B - less-well designed RCT or high quality cross-sectional study with valid ROM techniques as well as published reliability of technique that found an association, C - Less-well designed cross-sectional study with reliability not published or ROM technique that has not been validated. D - Poorly designed study or no association.
Adapted level of certainty [44] for cross-sectional studies: High - available evidence indicates consistent results from available well-conducted studies, Moderate - available evidence is sufficient to determine effects of association, although studies may be limited by various factors, such as methods, sample size, reliability of methods, etc.
Low - available evidence is insufficient to assess the association.

Table A.2 Summary of intrinsic factors associated with ROM, as well as a level of certainty of association [44].

Factor investigated	Authors, journal and year of publication	Type of study and (level of evidence) [43]	Population studied	Joint/structure	Method of investigation	Conclusion	Assessment of study	Overall level of certainty for factor
Age	Barnes, 2001, J shoulder elbow Surg. [66]	Cross-sectional (III)	General population (M+F)	Shoulder	Forward elevation, abduction, internal and external rotation, extension. Goniometer – randomized order to minimize warming-up effect.	Internal rotation ROM increases sig., ($p<0.01$) with age. All other measures decrease sig. with age.	C	HIGH
	De Araujo, 2008, Arq Bras Cardiol [21]	Cross-sectional (III)	General population (M + F) – mainly Caucasian (author-determined)	20 body joints	Flexitest – score of between 0 and 4 allocated to each limb.	ROM decreases with age in both male and females	C	
	Gabbe et al., 2006, J Sci Med Sport [10]	Cross-sectional (III)	Community- and elite-level Australian Rules Football players (M?)	Hamstring, quadriceps and iliopsoas flexibility; lumbar spine, dorsiflexion and hip rotation ROM; neural mobility	AKE, passive SLR, SR test (standard), dorsiflexion lunge test, active hip rotation, Modified Thomas Test, active slump test.	Older players (>25 years) had sig. ($p<0.05$) less ankle dorsiflexion ROM than younger players,	C	

						but NO difference in hamstring ROM.		
	Sullivan, 1994, Spine [67]	Cross-sectional (III)	Apparently healthy subjects, 15-65 yrs old (M + F)	Lumbar sagittal ROM	Flexion and extension (and the total of these). Measured with fluid-filled inclinometer.	Flexion, extension and total sagittal ROM decreased sig. with increasing age.	C	
	Youdas et al., 2005, JOSPT [68]	Cross-sectional (III)	Healthy adults, 20-79 yrs old (M + F)	Hamstring ROM	Passive SLR and popliteal angle - order randomized. Right side always tested first. Goniometer use. Very high ICC for measurements.	Age did not have a sig. effect on straight leg raise or popliteal angle measures.	C	

	Peate et al., 2007, J Occup Med Tox [72]	Cross-sectional (III)	U.S firefighters, 21-60 years old (M + F)	Trunk ROM.	Functional Movement Screen – 7 assessments. Active SLR for trunk.	Score decreased sig. ($p<0.001$) with increasing age.	C	
Gender	Wang, 1993, JOSPT [27]	Case-control (III)	Long-distance runners and non-running controls (M + F).	Hamstrings, quadriceps and posterior calf muscles.	Modified straight leg raise, modified Thomas test, ankle dorsiflexion	Females have significantly ($p<0.05$) more hamstring ROM than males.	B	HIGH
	Mafulli, 1994 BJSM [53]	Observational {III}	Elite young athletes, aged 9-18 years old practicing football, gymnastics, swimming and tennis (M + F)	Shoulder, trunk, "front split", quadriceps.	Validated linear measures of muscle stiffness. Better of two attempts recorded. Following 2min warm-up.	Female gymnasts sig. ($p<0.03$) more ROM than males in upper limbs.	C	
	Barnes, 2001, J shoulder elbow Surg [66]	Cross-sectional (III)	General population (M+F)	Shoulder	Forward elevation, abduction, internal and external rotation,	Females have sig. ($p<0.01$) greater ROM than males in all	C	

					extension. Goniometer – randomized order to minimize warming-up effect.	measures.		
	de Araujo, 2008, Arq Bras Cardiol [21]	Cross-sectional (III)	General population (M + F) – mainly Caucasian (author-determined)	20 body joints	Flexitest – score of between 0 and 4 allocated to each limb.	Females sig. more ROM than males as of 5 years of age. This is intensified post-puberty.	C	
	Kibler and Chandler, 2003, J Sci Med Sport [73]	RCT (I)	Junior tennis players enrolled in United States Tennis Association (M + F)	Hamstring, Gastrocnemius, Quadriceps, ITB, Hip ROM, Shoulder, Forearm, Wrist.	SR test, hip flexion, shoulder rotation, forearm pronation/ Supination, wrist flexion/ Extension.	Young females sig. more ROM than males at IT Band, Hip internal rotation, forearm supination ($p<0.05$) and hamstrings ($p<0.01$).	B	
	Sullivan, 1994, Spine [67]	Cross-sectional (III)	Apparently healthy subjects, 15-65 yrs old (M	Lumbar sagittal ROM	Flexion and extension (and the total of these). Measured with fluid-filled inclinometer.	Extension and total sagittal ROM sig. greater in females.	C	

			+ F)					
	Youdas et al., 2005, JOSPT [68]	Cross-sectional (III)	Healthy adults, 20-79 yrs old (M + F)	Hamstring ROM	Passive SLR and popliteal angle - order randomized. Right side always tested first. Goniometer use. Very high ICC for measurements.	Males sig. ($p<0.001$) less flexible than females. In both measures.	C	
Limb dominance	Wang, 1993, JOSPT [27]	Case-control (III)	Long-distance runners and non-running controls (M + F)	Hamstrings, quadriceps and posterior calf muscles.	Modified straight leg raise, modified Thomas test, ankle dorsiflexion	Non-dominant hamstring had sig. ($p<0.05$) more ROM than dominant leg	B	HIGH
	Barnes et al., 2001, J shoulder elbow Surg [66]	Cross-sectional (III)	General population (M+F)	Shoulder ROM	Forward elevation, abduction, internal and external rotation, extension. Goniometer – randomized order to minimize warming-up effect.	Non-dom shoulder had sig. ($p<0.05$) greater internal rotation and extension, but sig. ($p<0.01$) less external rotation than dom. shoulder.	C	

	Mafulli, 1994 BJSM [53]	Cross-sectional (III)	Elite young athletes, aged 9-18 years old practicing football, gymnastics, swimming and tennis (M + F)	Shoulder, trunk, “front split”, quadriceps.	Validated linear measures of muscle stiffness. Better of two attempts recorded. Following 2min warm-up.	Right shoulder side sig. more flexible than left ($p<0.05$) in all sports.	C	
	Macedo and Magee, 2008, J Manip. Physiol. Ther. [75]	Cross-sectional (III)	White university students and staff, 18-59 years old (F).	Upper and lower extremity ROM.	Validated methods with high ICCs. Goniometer used. No warm-up allowed, all performed within 2 hours.	34 of the 60 ROM measures were sig. different between dom. and non-dom. sides. 24 of these 34 were greater on the non-dom. side.	B	
	Gunal et al., 1998, JBJS-Am [100]	Cross-sectional (III)	Healthy military volunteers, 18-22 yrs (M).	Shoulder, elbow, forearm and wrist.	Hip extension, flexion, abduction, adduction, int. and ext rotation. Knee flexion and extension. Ankle extension, flexion,	Almost all measures sig. different between sides. Non-dom side always sig. more ROM.	C	

					valgus and varus. Used goniometer.			
	Roaas and Andersson, 1982, JBJS-Am [76]	Cross-sectional (III)	Healthy sample, 30-40 years old (DNS gender)	Hip, knee and ankle ROM	Hip extension, flexion, abduction, adduction, int. and ext rotation. Knee flexion and extension. Ankle extension, flexion, valgus and varus. Goniometer used.	No sig. difference between right and left sides.	D	
	Baltaci et al., 2001, J Sports Med Phys Fit [74]	Cross-sectional (III)	Collegiate baseball players, 18-22 yrs old (M)	Shoulder ROM	Active and passive int. and ext. rot. with goniometer. Non-dom. measured before dom. Ext. rot. measured before int. rot.	Sig. ($p<0.05$) more ext. rot. and less int. rot in dominant shoulder compared to non-dominant in pitchers.	B	
	Conte et al., 2009, J Manip Phys Ther. [77]	Cross-sectional (III)	Non-athletes 20-27 yrs old (F)	Shoulder ROM	Passive flexion, horizontal adduction, extension, int. and ext rotation.	Sig. ($p<0.05$) more ext rot and less int. rot in dominant shoulder	B	

					Measured using goniometer. High ICC for all measures	compared to non-dominant shoulder.		
Prior injury	Peate et al., 2007, J Occup Med Tox [72]	Cross-sectional (III)	U.S firefighters, 21-60 years old (M + F)	Trunk ROM.	Functional Movement Screen – 7 assessments. Active SLR assessed trunk flex.	Those with prior injury did not score sig. less.	D	MODERATE
	Jonhagen et al., 1994, AJSM [59]	Retrospective comparative (III)	Recently injured (hamstring) vs. injury-free sprinters (male)	Hip joint ROM.	Passive SLR test. No warm-up before test.	Recently injured sprinters had sig. ($p<0.05$) less hamstring ROM than uninjured sprinters.	C	
	Henderson et al. , 2009, J Sci. Med. Sport [49] .	Retrospective comparative (III)	Elite soccer players (male)	Hip flexion/hamstring ROM	Active and passive SLR following standardized warm-up	Both active and passive SLR were higher in non-injured, although not significantly so.	D	
	Heiderscheit et al., 2005, Clin. Biomechanics [79]	Case study (IV)	31 year old professional skier (M)	Hip flexion, knee extension.	Motion analysis from reflective markers.	Hip and knee joint angle decreased very slightly following injury	C	

	Askling et al., 2006, BJSM [80]	Retrospective comparative (III)	Eighteen elite sprinters and 15 dancers who recently suffered an acute hamstring injury.	Hip flexion	Passive SLR without a warm-up.	Injured leg significantly less ROM than uninjured leg at 2, 10, 21 and 42 days after injury.	C	
Weight/BMI	Miyatake et al., 2001, Chin Med Journal [82]	Cross-sectional (III)	Normal (71), borderline obese (71) and obese Japanese (71) population (M).	Hip joint ROM.	SR test (standard)	Weight ($p<0.05$) and BMI ($p=0.05$) negatively correlated with ROM in obese subjects.	B	MODERATE
	Mason et al., 2007, MSSE [81]	Prospective cohort (II)	General Canadian population, 20-69 years of age. (606 M + F)	Trunk flexibility	SR test (standard)	Baseline BMI sig. ($p<0.05$) negatively correlated with Hip ROM.	B	

	Gilleard and Smith, Int. J Obes [83]	Cross-sectional (III)	Obese females and age-matched non-obese females	Thorax, pelvis, hip and thoracolumbar spine	Standing and seated trunk flexion motion analysis system.	BMI sig. negatively correlated with thoracolumbar spine, but not hip ROM	C	
Height	Hahn et al., 1999, SJMSS [84]	Cross-sectional (III)	Active, non-competitive athletes, 14-24 years old (M+ F)	Knee ROM	Active knee extension and flexion test using goniometer. No physical exercise or stretching 30 min before test.	Height sig. negatively associated with both measures of knee ROM.	C	LOW
Muscle size	Magnusson et al., 1997, Scand J Med Sci Sport [85]	Cross-sectional (III)	Elite-level orienteers (M)	Hamstring ROM.	Toe-touch test	Lateral hamstring cross-sectional area sig. ($p < 0.05$) negatively related to toe-touch test.	C	LOW

Flexibility training	Hartig and Henderson, 1996, AJSM [25]	RCT (I)	Basic military recruits, average age of 20 years (M + F)	Hamstring ROM	Knee extension with hip flexed at 90° - assessed with Goniometer High reliability coefficient.	Regular passive stretching protocol significantly increased hamstring ROM.	A	HIGH
	Davis et al., 2005, J Strength Cond. Res. [89]	RCT (II)	Individuals with tight hamstrings (as determined by A.K.E test), 18-40 years of age.	Hamstring ROM.	A.K.E test using inclinometer - end ROM determined by subject.	Static stretching protocol resulted in sig. increase in hamstring ROM after 4 weeks of training (3x per week).	B	
	Hortobagyi et al., 1985, Int. J Sports Med [121].	RCT (I)	Healthy, active secondary school children (M)	Lower body ROM.	Front splits, supine leg pull and split while supine, Dynamometer used to assess ROM	ROM improved sig. in all three measures after 7 weeks of training.	B	

	Bandy et al., 1998, JOSPT [88]	RCT (I)	Fifty-eight subjects, 22-46 years old. Without soft-tissue pathology but tight hamstrings (A.K.E test).	Hamstring ROM.	A.K.E test as described by Norkin and White [18] without a warm-up. Goniometer used for measurement. High ICC for assessment	30s stretch 5 x per week for 6 weeks most effective to increase hamstring ROM.	A	
	Stanziano et al., 2009, Clin. Inter. Aging [122]	RCT (I)	Subjects were from a residential retirement community, average age 88.7 years (M + F).	Functional ROM	Back-scratch, Modified chair sit-and reach, supine knee extension and total body rotation tests	Stretching program 2x per week for 8 wks significantly improved ROM in all measures in comparison to a non-stretching control group	B	

	Medina et al., 2007, J Sports Med. Phys Fit. [91]	RCT (I)	Schoolchildren – aged 10-11 years.	Hip ROM	SLR according to AAOS - end range determined by tester or visible pelvic rotation. Same time of day and temp. No prior warm-up permitted.	Stretching groups both had sig. increase in hip ROM. Control group had a non-sig. decrease in hip ROM.	B	
Genotype	Collins et al., 2008, SJMSS [97]	Cross-sectional (III)	Achilles rupture and tendinopathy groups, along with apparently healthy adults (M + F)	Hamstring and lower back ROM.	Sit and reach and Straight Leg raise test (latter performed with goniometer). Measures performed without warm-up. High ICC values for tests.	Heterozygotes sig. less flexible than homozygotes of BstUI genotype.	C	LOW

BJSM – British Journal of Sports Medicine; SJSMM – Scandinavian Journal of Medicine and Science in Sport; AJSM – American Journal of Sports Medicine; MSSE – Medicine and Science in Sports and Exercise; SR - sit and reach; SLR – Straight Leg Raise; A.K.E – active knee extension; RCT – Randomized Control Trial; ROM – range of motion. AAOS - American College of Orthopaedic Surgeons
Adapted magnitude of net benefit: [44] for cross-sectional studies: A - Well designed RCT that found an association, B - less-well designed RCT or high quality cross-sectional study with valid ROM techniques as well as published reliability of technique that found an association, C - Less-well designed cross-sectional study with reliability not published or ROM technique that has not been validated. D - Poorly designed study or no association.

Adapted level of certainty [44] for cross-sectional studies: High - available evidence indicates consistent results from available well-conducted studies, Moderate - available evidence is sufficient to determine effects of association, although studies may be limited by various factors, such as methods, sample size, reliability of methods, etc.
Low - available evidence is insufficient to assess the association

Appendix B - Ethics approval letter 2008

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
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05 February 2009

REC REF: 092/2008

A/Prof M Collins
Human Biology

Dear A/Prof Collins

PROJECT TITLE: GENOTYPE EFFECTS ON RANGE OF MOTION MEASUREMENTS IN HEALTHY INDIVIDUALS.

Thank you for your letter to the Research Ethics Committee dated 28th January 2009.

It is a pleasure to inform you that the Ethics Committee has **approved** the Amendments listed below with reference to the above-mentioned study:-

- Increase the sample size from 200 to 400 participants.
- Broaden the age range of the participants from 18 to 50 years.
- Add a second 15 minute visit to the protocol where one of the range of motion measurements will be done.

Annual approval will be granted after an annual report.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

lemjedi

Appendix B2 - Ethics approval letter 2009

UNIVERSITY OF CAPE TOWN

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05 February 2009

REC REF: 092/2008

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Human Biology

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Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

lemjedi

Appendix C - Informed consent

THE GENETIC EFFECTS ON FLEXIBILTY MEASUREMENTS

INFORMED CONSENT

I, _____ (the participant), have been fully informed about this study on the genetic basis of flexibility measurements to be conducted by the UCT/MRC Research Unit for Exercise Science and Sports Medicine at the University of Cape.

I have agreed to donate five millilitres of venous blood, which will be used for the extraction and analysis of genetic material (DNA), and will be taken by a phlebotomist. The DNA will only be used for scientific research purposes relating to the genetic basis of flexibility measurements. I agree to my height, weight and waist circumference measured. I have also agreed to complete questionnaires relating to personal particulars, sporting participation, medical history, stretching and warm up exercise and understand that all the information that is collected during the study will be treated with the strictest confidentiality and will only be used for scientific research purposes. I also understand that all data will be analysed anonymously and my DNA sample will be destroyed on completion of the study. I understand that the DNA will be genotyped (analysed) for variations (polymorphisms) within the *COL5A1* and other similar genes relating to the genetic basis of flexibility measurements.

I am also prepared to have the flexibility of my upper and lower limbs measured. I am prepared to have my upper leg prepared for and have EMG electrodes attached to measure the activity in my muscles during the lower limb flexibility test. I have agreed to allow the researchers to do a standard stretching procedure to increase my hamstring and shoulder flexibility.

The potential risks associated with blood collection technique from the ante-cubital veins are: infection, delayed healing, haematoma, physical pain, mental discomfort and injury

to a nerve or a vessel. These risks are small and will be minimized by the use of trained phlebotomists, use of sterile techniques and the use of disposable, single use materials.

I understand that whilst there is no direct benefit to myself, a genetic predisposition for flexibility can be established. I have read (or, where appropriate, have had read to me) and understood the information about this study, and any questions I have asked have been answered to my satisfaction. I agree to participate in the study, realising that I have the right to request that my DNA sample be destroyed at anytime and, further, to demand that data arising from my participation is not used in the research project provided that this right is exercised within four weeks of the completion of my participation in the project. I agree that research data provided by me or with my permission during the project may be included in a thesis, presented at conferences and published in journals on the condition that neither my name nor any other identifying information is used.

Any questions regarding this project may be directed to the Principle Investigator: **Prof Malcolm Collins** on telephone number **021 650 4574** or e-mail **malcolm.collins@uct.ac.za**.

If you have any complaints or queries that the investigator has not been able to answer to your satisfaction, you may contact the Faculty of Health Sciences Human Research Ethics Committee at the University of Cape Town **Prof Marc Blockman** on telephone number **021 406 6452**.

Name of Participant: _____

Signature: _____

Date:_____

Name of Researcher:_____

Signature: _____

Date:_____

Appendix D1 – Medical and training questionnaire for running events

Department of Human Biology

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2009 MR PRICE WINELANDS MARATHON – MEDICAL AND TRAINING QUESTIONNAIRES

These questionnaires have been constructed by the Medical Research team of the Exercise Science and Sports Medicine Unit. The information obtained from these questionnaires is essential for the planning of medical care during events such as the Mr Price Winelands Marathon. We acknowledge that the questionnaires are long, but we are asking about 30 minutes of your valuable time to complete them. The completion of the questionnaires is voluntary; all the information will be kept confidential and will only be used for research and medical care planning purposes. We suggest that you consider downloading and completing this before the event and handing in the completed questionnaire, at the research area during race registration.

Prof Martin Schwellnus (Chairman, Research Team)

Instructions

Please answer each question by filling in the details in the allocated space or checking one or more of the option boxes.

Please bring the completed forms together with the signed consent form to the research table at race registration.

Please complete sections A, B, C, D, E and F

Section A	Personal Details	Page 2
Section B	Racing, Training and Equipment Use History	Pages 3-5
Section C	History of Medication, Supplement and Fluid Use as well as Lifestyle and Habits History	Pages 5-7
Section D	Family Medical History	Page 8
Section E	General Personal Medical History	Pages 9-11

Please complete only the relevant questions in the following section

Section F	Additional Detailed Medical History	Pages 12-23

Section A: Personal details			
2009 Race Number			
Surname			
First Name			
Postal Address		Postal/ Zip Code	
E-mail address		Phone (day time)	code number
Alternate E-mail address			
Date of birth	yyyy-mm-dd	Cell (Mobile)	
Height	cm	Gender	Male ☐ Female ☐
Weight	kg	Age (on race day)	yrs
Ethnic group (Only Required and Used for Research Purposes)	Black/African ☐ White ☐ Indian ☐ Mixed Ancestry (Coloured) ☐ Asian ☐ Other ☐		
Ancestry: Tribal or national background	Father:		Unknown ☐
	Mother:		Unknown ☐
Country of Birth			
Dominant Hand	Left ☐ Right ☐ Both ☐	Dominant Leg	Left ☐ Right ☐ Both ☐
Occupation			
What **percentage** of your **working** day is spent in the following activities?	Sitting: _____ % Standing: _____ % Walking (Lower body activity) _____ % Manual Labour (upper and body activity) _____ %		

Section B. Racing and training history			
Type of running event	**10 km**	**21.1 km**	**42.2 km**
Which road running races have you **ever** participated in?	Yes ☐ No☐	Yes ☐ No ☐	Yes ☐ No ☐
Year of first event			
How many of these events have you **ever** participated in?			
Personal best time **ever**	_____ min	_____ min	_____ min
What is your best time, in a running race, in the **last 15 weeks**?	_____ min	_____ min	_____ min
Type of event	**Two Oceans Ultra-Marathon**	**Comrades Marathon**	
Which races have you **ever** participated in?	Yes ☐ No☐	Yes ☐ No ☐	
Year of first event			
How many events have you **ever** participated in?			
Personal best time	_____ hrs:min	_____ hrs:min	
What is your predicted time for the 2009 Mr Price Winelands Marathon?	_____ hrs _____ min		

Please answer the following questions, with your answers reflecting your average in the **most recent 15 weeks i.e. 2nd week of August 2009 to 20th November, 2009.**	
Do you train with a heart rate monitor?	Yes ☐ No☐
Do you race with a heart rate monitor?	Yes ☐ No☐
Do you use heart rate information to control your training pace?	Yes ☐ No☐
Do you use heart rate information to control your racing pace?	Yes ☐ No☐
Do you record, download and store your heart rate information?	Yes ☐ No☐
Would you be willing to make your heart rate data available to the research team?	Yes ☐ No☐
How many days a week did you train during the **last 15 weeks**?	days/wk
What distance did you train in an average week during the **last 15 weeks**?	km/wk

How many hours a week did you **train** in an average week during the **last 15 weeks**?	hrs/wk
How many hours a week did you **work** in an average week during the **last 15 weeks**?	hrs/wk
What **distances** did you train in the week before the race?	km
How many **hours** did you train in the **week before** the race?	hours
How many fast/ hard sessions did you do per week in the **last 8 weeks**?	
Describe briefly the session, including distance, time and recovery interval (if applicable) e.g. 10 x 400m in 75 sec with 60 sec jog recovery between each	
What percentage of your weekly training distance was done at race 42.2 km speed or faster?	%
How many hours did you train 3 days before the race	hours
How many hours did you train 2 days before the race	hours
How many hours did you train the day before the race	hours
How did your training commitment affect your social life?	☐ Not at all ☐ A fair amount ☐ A lot

Flexibility training history	
Do you perform flexibility training (regular stretching exercises)?	Yes ☐ No ☐
If YES, please complete the rest of the flexibility training history section below:- If NO, continue completing the questionnaire from the top of page 5 (Equipment use history).	
On average, how many days a week do you perform a stretching session?	days/week
On average, how times a day do you perform a stretching session?	times/day
Please tick which muscle groups do you include in your stretching session?	☐ Hamstrings ☐ Quadriceps ☐ Calf (gastrocnemius) ☐ Calf (soleus) ☐ Groin (inner thigh) ☐ Upper body limbs ☐ Other: _____

Please tick when you stretch? (before, during and/or after exercising. You can tick more than one box)	☐ Before Exercise ☐ During Exercise ☐ After Exercise
When you stretch an individual muscle group, on average, **how long do you hold the stretch** for?	seconds
When you stretch an individual muscle group, on average, **how many times do you stretch the muscle for**?	☐ Once ☐ Twice ☐ 3 times ☐ 4 times ☐ 5 times ☐ 6 or more times

Equipment use history	
Please indicate which **brand of running shoe** you use?	☐ Adidas ☐ Asics ☐ Brooks ☐ New Balance ☐ Nike ☐ Mizuno ☐ Puma ☐ Reebok ☐ Saucony ☐ Other: _____
Please indicate which **type of running shoe** you use?	☐ Soft neutral shoe ☐ Mild anti-pronation shoe ☐ Motion control shoe ☐ Light racing shoe ☐ Unknown or not sure ☐ Other: _____

Section C. History of medication and supplement use		
What medication, if any, are you currently using? (please list)	Name of medication	Years taken
Do you use protective skin sunscreen during training session or when competing?	Yes ☐ No ☐	☐ Every session ☐ Most sessions ☐ Some sessions ☐ Very occasionally
Are you currently taking dietary supplements/vitamins?		Yes ☐ No ☐
If **yes** to the above question, please list names of dietary, sports or vitamin supplements.	Name of supplement	Years taken
	☐ Multi-vitamins ☐ Anti-oxidants ☐ Immune boosters ☐ Protein powders/supplements, Protein bars. BCAAs ☐ Creatine ☐ Caffeine ☐ Fat cutters ☐ Carbohydrate drinks/powders/gels ☐ Other: _____	_____ _____ _____ _____ _____ _____ _____ _____
Have you ever used oral corticosteroids (cortisone tablets)? (If **yes**, how long ago?)	Yes ☐ No ☐	☐ 3 months ☐ 6 months ☐ 12 months ☐ 24 or more months
Have you ever been given an injection with corticosteroids? (If **yes**, how long ago?)	Yes ☐ No ☐	☐ 3 months ☐ 6 months ☐ 12 months ☐ 24 or more months
Have you ever been given an injection of corticosteroids in or around the **Achilles** tendon? (If **yes**, how many times?)	Yes ☐ No ☐	☐ Once ☐ Twice ☐ 3 times ☐ >3 times
Have you ever used fluoroquinoline antibiotics? (refer to the following list)	Yes ☐ No ☐	☐ 3 months ☐ 6 months ☐ 12 months ☐ 24 or more months

List of some fluoroquinoline antibiotics:		
ADCO-CIPRIN	CIPROBAY	SANDOZ CIPROFLOXACIN
AVELON	CIPROGEN	TAFLOC
BACTIDRON	CPL ALLIANCE CIPROFLOXACIN	TARIVID
CIFLOC	DYNAFLOC	TAVANIC
CIFRAN	FACTIVE	TEQUIN
CIPLA-CIPROFLOXACIN	FLOXIN	UNIQUIN
CIPLOXX	MAXAQUIN	UTIN-400
CIPRO-HEXAL	NOROXIN	ZANOCIN
	ORPIC	

Lifestyle and habits history			
Please indicate your smoking status	Current smoker ☐	Ex smoker ☐	Never smoked ☐
If you answered yes, (past or current smoker) please complete the section on the right	Number of years of smoking:	If stopped, how many years ago:	
	What is (was) the average number of cigarettes per day:		
On average, how much alcohol do you drink per week (tots, glasses) of spirits, wine or beer?	_____ glasses beer/cider per week _____ glasses wine per week _____ tots of spirits per week		

Fluid Intake	
How do you best describe your fluid intake during an Ironman triathlon race?	(a) I drink to thirst ☐ (b) I drink as much as tolerable ☐ (c) I drink according to a predetermined fluid intake schedule ☐ (d) I drink to prevent any weight loss during exercise ☐ (e) I combine (a) with (c) ☐ (f) I combine (b) with (c) ☐ (g) Other: _____ ☐
What percentage of your fluid intake will consist of these beverages?	Water: ☐ 0-25% ☐ 26-50% ☐ 51-75% ☐ 76-100% Sports drink: ☐ 0-25% ☐ 26-50% ☐ 51-75% ☐ 76-100% Coke: ☐ 0-25% ☐ 26-51% ☐ 51-75% ☐ 76-100% Other: ☐ 0-25% ☐ 26-50% ☐ 51-75% ☐ 76-100% Specify other: _____
What will be your estimated **total** fluid intake be **(if at all)** during the **swim**?	ml
What will be your estimated **total** fluid intake be during the **cycle**?	ml
What will be your estimated **total** fluid intake be during the **run**?	ml

Rank the following sources of information on their importance in formulating your drinking strategy. (1 being most influential and the lowest number being least influential)	_____ Fellow triathletes _____ Coach / trainer _____ Magazines / books _____ Website (please specify: _____) _____ Drinking guidelines from sports associations _____ Adverts _____ Self-experimentation _____ Other: _____

Section D. Family medical history		
Have any of your blood (biological) relatives <u>ever</u> had the following? **Please tick yes or no. If yes, please tick the relationship of that person to you (You may tick more than one of the relationship blocks).**		
Description		**If Yes, please indicate the relationship**
Exercise associated muscle cramps	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Night muscle cramps	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Chronic Achilles tendon injury	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Achilles tendon rupture	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Any ligament injury	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Asthma	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Allergies (in general)	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Heart Disease	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Diabetes	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Depression, Anxiety attacks, Personality disorder	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Gastro-intestinal (GIT) disease	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother

Section E. Personal general medical history	
In this section, you are asked to read through 14 questions about your personal general medical history. If you answer "yes" to any of questions 1 to 12, please complete the additional questions at the end of the section (section G on page 18).	
1. In the **6 weeks before this race** (from 9th October) did you suffer from any **symptoms of flu** (fever, sore throat, blocked or runny nose, cough, wheeze, muscle aches and pains)?	Yes ☐ No ☐
2. Have you **ever** in your running career suffered from **muscle cramping** (painful, spontaneous, sustained spasm of a muscle) during or immediately (within 6 hours) after exercise (in training or competition)?	Yes ☐ No ☐
3. Have you **ever** in your triathlon career suffered from **a tendon or ligament injury** (pain, swelling, stiffness) in any tendon (including Achilles tendon, knee tendons, and shoulder tendons) or ligaments (partial or complete tear)?	Yes ☐ No ☐
4. Have you **ever** in your running career **used medicines to treat injuries** in the week **before or during a race** – including anti-inflammatory drugs, cortisone (pills, or injection), or pain killers?	Yes ☐ No ☐
5. Have you **ever** in your running career suffered **gastrointestinal** symptoms **during exercise** including heartburn, nausea, vomiting, abdominal pain, urge to defecate (pass a stool), diarrhoea, or blood in the stools?	Yes ☐ No ☐
6. Have you **ever** in your running career suffered from symptoms of the **nervous system** including exercise induced headaches, nerve tingling or loss of sensation?	Yes ☐ No ☐
7. Have you **ever** in your running career suffered from **symptoms of allergies** including nose allergies (hay fever), allergic sinusitis, allergic asthma, skin allergies, a past history of allergies to medication, plant material or animal material?	Yes ☐ No ☐
8. Do you **currently suffer from asthma** including exercise induced asthma, or symptoms of asthma such as shortness of breath, wheezing, or chronic coughing?	Yes ☐ No ☐
9. Have you ever **collapsed** (fell down **not because of an accident,** needing medical attention) during, at the finish or after a race or training session?	Yes ☐ No ☐
10. Do you **currently** suffer from any **symptoms of injury** in the muscles, tendons, bones, ligaments or joints?	Yes ☐ No ☐

11. Do you **currently**, or did you **in the last year**, suffer from any symptoms of **exercise related skin disease**?	Sunburn: Yes ☐ No ☐ Skin cancer: Yes ☐ No ☐ Other skin damage resulting sun exposure: Yes ☐ No ☐
12. Please tick in which anatomical area you ever had **surgery** performed.	☐ Gastric (stomach) ☐ Oesophageal (swallowing pipe) ☐ Small bowel ☐ Large bowel (colon) ☐ Rectum ☐ Gallbladder ☐ Pancreas ☐ Liver ☐ Abdomen (general) ☐ Wrist ☐ Head ☐ Finger ☐ Neck ☐ Lower back ☐ Face ☐ Hip ☐ Front chest ☐ Thigh ☐ Back chest ☐ Knee ☐ Shoulder ☐ Lower leg ☐ Upper arm ☐ Achilles ☐ Elbow ☐ Ankle ☐ Forearm ☐ Foot ☐ Other (Specify: _____)
13. Management of pain during the last 3 months	
14a. Did you alter or stop your training schedule due to pain in any part of your body?	Yes ☐ No☐
If yes: For how long	_____ days
Did you adapt your training schedule for a while when your injury/illness was healed?	Yes ☐ No☐
14b. How do you feel when you experience pain? (you can tick more than one option)	☐ It does not bother me much ☐ Angry ☐ Frustrated ☐ Depressed ☐ Resentful ☐ Overwhelmed

14c.When you experience pain, do you? (you can tick more than one option)	☐ Adjust your training schedule ☐ Stop training ☐ Slowly get "back on track" of your training schedule ☐ Train harder to make up for the missed training sessions ☐ Ignore the pain and continue to train ☐ Feel scared to do anything that could aggravate the pain ☐ Think that the pain means that you have a severe injury ☐ Tell everybody about it
14. **Female athletes only:** Please complete the following questions (14a. to 14g.) related to your menstrual cycle and other gynaecological history	
15a. At what age did you start your periods (menstruating)?	(years)
15b. <u>In the last 12 months</u>, how many menstrual cycles did you have?	
15c. Have you ever had irregular menstrual periods in the past? (excluding pregnancy)?	Yes ☐ No ☐
15d. Have you had a hysterectomy/ovarectomy?	Yes ☐ No ☐
15e. How many times have you been pregnant?	(times)
15f. What form of contraception are you currently using?	☐ None ☐ Oral contraceptive pill ☐ Injection ☐ Intra-uterine device ☐ Sterilization (tubes tied) ☐ Other: _____
15g. If yes to question 15f. above, for <u>oral contraceptive pill</u>, for what reason was the pill prescribed?	☐ Not applicable ☐ Dermatological ☐ Contraception ☐ Regulate period ☐ Other: _____

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

<u>If you have answered **YES** to any of the first 11 questions of the Personal General Medical History questionnaire (section E) please complete the relevant additional questions that follow in section F.</u>

Please bring the completed forms together with the signed consent form to the pre-race facility or the research table at race registration.

Section F. Additional detailed medical history ***(Please complete all the sections to which you answered "Yes" in the Personal general medical history)***

1. Flu symptoms in the last 6 weeks If you answered **YES** to **question 1** in section E, please complete the following two questions related to flu symptoms in the last 6 weeks.	
(1a) Please tick which of these flu symptoms you suffered from **in the last 6 weeks**.	☐ Fever ☐ Cough ☐ Joint pains ☐ Blocked nose ☐ Wheezing ☐ Sore Throat ☐ Runny nose ☐ Muscle aches ☐ Any other flu symptoms (Specify: _____)
(1b) Please tick which of these flu symptoms you suffered from **in the last 7 days**.	☐ Fever ☐ Cough ☐ Joint pains ☐ Blocked nose ☐ Wheezing ☐ Sore Throat ☐ Runny nose ☐ Muscle aches ☐ Any other flu symptoms (Specify: _____)

2. Muscle cramping If you answered **YES** to **question 2** in section E, please complete the following questions (2a. to 2m.) related to your cramping.	
(2a) For how many years have you suffered from cramping?	(years)
(2b) Did you suffer from cramping during or after exercise in the **last 12 months**?	Yes ☐ No ☐
(2c) With what **type of exercise** is your cramping associated (You can tick more than one form of exercise)?	☐ Swimming ☐ Cycling ☐ Running
(2d) In the **last 10 races or training sessions**, how many times have you experienced cramping?	Races: _____/10 Training sessions: _____/10

(2e) What treatment/s have you had that **successfully relieved** an acute cramp? (can tick more than one)	☐ Stretching ☐ Resting ☐ Drinking fluid ☐ Ice application ☐ Massage ☐ Magnesium ☐ Salt (tablets or solution) ☐ Other (Specify: _____)
(2f) At **what point in the race or training run** do you usually first experience cramping?	☐ First quarter ☐ Second quarter ☐ Third quarter ☐ Fourth quarter ☐ After the race ☐ No pattern
(2g) In which **muscles** do you usually cramp (please list the muscle by the one which cramps most frequently (as 1) and the others after that (2-4)?	☐ Calves ☐ Hamstrings ☐ Quadriceps (thigh) ☐ Foot muscles ☐ Other (Specify: _____)
(2h) Have you **ever** suffered from cramping in your **whole body** (arms and legs)?	Yes ☐ No ☐
(2i) Have you **ever** been **admitted to hospital** following cramping?	Yes ☐ No ☐
(2j) Have you **ever** been **confused or in a coma** during or after a cramping episode?	Yes ☐ No ☐
(2k) Have you ever had **"dark urine"** in the 3 days following a cramping episode?	Yes ☐ No ☐
(2l) If you cramp, **how long** does the cramp usually last for (min)?	(minutes)
(2m) If you cramp, how **severe** is the cramp usually? (please tick).	☐ Mild: < 5 minutes and you are able to continue exercising ☐ Moderate: 5-15 minutes and you are able to continue exercising ☐ Severe: >15 minutes or if you have to STOP exercising

3. Past Tendon and Ligament Injury History

If you answered **YES** to **question 3** in section E, please complete the following questions (3a. to 3d.) related to your past history of tendon/ligament injury/ies.

(3a) Please tick which **tendon/s** you have injured? (next column on the tright)		**Tendon**	**Longstanding Pain (Tendinopathy)**	**Acute Tear/ Rupture**
Also indicate (tick) if your injured	Foot and ankle:	☐ Achilles tendon	☐	☐
		☐ Tibialis posterior	☐	☐
		☐ Plantar fascia	☐	☐

tendon was longsatnding pain (tendinopathy) or an acute tear/rupture	Knee:	☐ Patellar tendon	☐	☐
	Elbow and wrist:	☐ Wrist extensor tendon	☐	☐
	Shoulder:	☐ Rotator cuff	☐	☐
	Other: _____		☐	☐

(3b) Please tick which **ligament/s** you have injured? (next column on the tright) Also indicate if your sprained or completely tore the ligamant.	**Ligament**	**Sprain**	**Complete Tear**
	☐ Shoulder ligaments	☐	☐
	☐ Elbow ligaments	☐	☐
	☐ Wrist ligaments	☐	☐
	☐ Finger ligaments	☐	☐
	☐ Knee (ACL)	☐	☐
	☐ Knee (MCL)	☐	☐
	☐ Knee (PCL)	☐	☐
	☐ Knee (LCL)	☐	☐
	☐ Ankle lateral ligaments	☐	☐
	☐ Ankle medial ligaments	☐	☐
	☐ Spinal ligaments	☐	☐
	☐ Other: _____	☐	☐

(3c) Please tick if you have you ever suffered from any of the following **joint capsule** injuries?	☐ Acute shoulder dislocation ☐ Chronic shoulder instability ☐ Other: _____
(3d) Do you suffer from any other **connective tissue or rheumatological diseases** or disorders? (If yes, please specify which one)	Yes ☐ No ☐ (refer to the list on the next page) (If yes, specify: _____)

List of some Connective Tissue and/or Rheumatic Diseases and Disorders		
Ankylosing Spondylitis	Lipid Storage Diseases	Pseudogout
Aspartylglycosaminuria (AGU)	Marfan Syndrome	Reactive Arthritis
Behcet's Syndrome	Menkes Kinky Hair Syndrome	Reiter's Syndrome
Crohn's Disease	Mucopolysaccharidoses	Relapsing Polychrondritis
Discoid Lupus Erythematosus	Myopathies and Dystrophies	Scleroderma
Ehlers-Danlos syndrome (EDS)	Ochronosis (Homocystinuria)	Sjogren's Syndrome
Eosinophilic Fascitis	Osteogenesis imperfecta (OI)	Systemic Lupus Erythematosus (SLE)
Giant Cell (Temporal) Arthritis	Polyarteritis Nodosa	Systemic Sclerosis
Gout	Polymyalgia Rheumatica	Wegener's Granulomatosis
Hypersentive Vasulatis	Polymyositis & Dermatomyositis	

4. Use of medicines to treat an injury before or during participation If you answered **YES** to **question 4** in section E, please complete the following two questions related to medicine use for injuries before or during races.	
(4a) Which of the following medicines have you used in the past to treat an injury **in the week just before** a race?	☐ Paracetamol (e.g. Panado, Tylenol) ☐ Non-steroidal anti-inflammatories (e.g. Voltaren, Cataflam) ☐ Cortisone (pills) ☐ Cortisone injection ☐ Codeine ☐ Anti-inflammatory gels/creams/patches ☐ Any other pain killers (Specify: _____)
(4b) Which of the following medicines have you used in the past to treat an injury **during a race**?	☐ Paracetamol (e.g. Panado, Tylenol) ☐ Non-steroidal anti-inflammatories (e.g. Voltaren, Cataflam) ☐ Cortisone (pills) ☐ Cortisone injection ☐ Codeine ☐ Anti-inflammatory gels/creams/patches ☐ Any other pain killers (Specify: _____)

5. Gastrointestinal symptoms during exercise

If you answered **YES** to **question 5** in section E, please indicate which gastrointestinal symptoms you have ever suffered from **during exercise** and, how frequently (in the last 12 months and in the last 10 races), and in which type of exercise.

Symptom	Number of times you experienced the GIT symptom in the last 12 months **(during exercise)**	Number of times you experienced the GIT symptom in the last 10 races **(during races)**	Please indicate which **type of exercise** is mostly associated with the GIT symptom	Please indicate the **"severity" of the GIT symptom** during exercise
Nausea			☐ Swimming ☐ Cycling ☐ Running	☐ Does not affect training or racing ☐ Affects training/racing (slow down or reduce time) ☐ Prevents training/racing
Vomiting			☐ Swimming ☐ Cycling ☐ Running	☐ Does not affect training or racing ☐ Affects training/racing (slow down or reduce time) ☐ Prevents training/racing
Heartburn			☐ Swimming ☐ Cycling ☐ Running	☐ Does not affect training or racing ☐ Affects training/racing (slow down or reduce time) ☐ Prevents training/racing
Abdominal pain			☐ Swimming ☐ Cycling ☐ Running	☐ Does not affect training or racing ☐ Affects training/racing (slow down or reduce time) ☐ Prevents training/racing
Urge to pass a stool (defecate)			☐ Swimming ☐ Cycling ☐ Running	☐ Does not affect training or racing ☐ Affects training/racing (slow down or reduce time) ☐ Prevents training/racing
Diarrhoea			☐ Swimming ☐ Cycling ☐ Running	☐ Does not affect training or racing ☐ Affects training/racing (slow down or reduce time) ☐ Prevents training/racing
Passing blood in the stool			☐ Swimming ☐ Cycling ☐ Running	☐ Does not affect training or racing ☐ Affects training/racing (slow down or reduce time) ☐ Prevents training/racing

Please indicate if you previously suffered from or had any of the following (you may tick more than one)?	☐ History of heartburn ☐ Gastroscopy ☐ Ulcer (gastric, duodenal) ☐ Irritable bowel syndrome ☐ Allergy to milk products ☐ Other past history of GIT disease

6. Diseases of the nervous system

If you answered **YES** to **question 6** in section E, please indicate which nervous disease symptoms you have ever suffered from **during exercise** and, how frequently (in the last 12 months and in the last 10 races), and in which type of exercise.

Symptom	Number of times in the last 12 months **(during exercise)**	Number of times in last 10 races **(during races)**	**Tick type of exercise**
Headaches			☐ Swimming, ☐ Cycling, ☐ Running
Nerve tingling in the hands			☐ Swimming, ☐ Cycling, ☐ Running
Loss of sensation in the hands			☐ Swimming, ☐ Cycling, ☐ Running

7. Allergy history If you answered **YES** to **question 7** in section E, please complete the following questions (7a. to 7e.) related to your current and past history of allergies.					
(7a) Please indicate how long (years) have you been suffering from allergies?					years
(7b) Please tick which <u>type of allergy</u> do you currently suffer from					
Nose (hay fever)	Yes ☐ No ☐	Sinusitis	Yes ☐ No ☐	Asthma (allergic)	Yes ☐ No ☐
Skin allergies	Yes ☐ No ☐	Eye allergies	Yes ☐ No ☐	Allergy to plant material	Yes ☐ No ☐
Allergy to foods	Yes ☐ No ☐	Allergy to animals	Yes ☐ No ☐	Allergy to medication	Yes ☐ No ☐
(7c) Please tick which <u>type of allergy</u> do you <u>currently take medication</u> for					
Nose (hay fever)	Yes ☐ No ☐	Sinusitis	Yes ☐ No ☐	Asthma (allergic)	Yes ☐ No ☐
Skin allergies	Yes ☐ No ☐	Eye allergies	Yes ☐ No ☐	Allergy to plant material	Yes ☐ No ☐
Allergy to foods	Yes ☐ No ☐	Allergy to animals	Yes ☐ No ☐	Allergy to medication	Yes ☐ No ☐
(7d) Please tick which <u>type of medication</u> do you <u>currently take</u>					
Cortisone nose spray	Yes ☐ No ☐	Cortisone nose inhaler	Yes ☐ No ☐	Anti-histamine tablets	Yes ☐ No ☐
Cortisone cream	Yes ☐ No ☐	Anti-histamine cream	Yes ☐ No ☐	Other inhaler / tablets or cream	Yes ☐ No ☐
(7e) Please tick which <u>symptoms of allergy</u> do you <u>currently suffer</u> from					
Sneezing	Yes ☐ No ☐	Itchy runny nose	Yes ☐ No ☐	Headache	Yes ☐ No ☐
Itchy palate	Yes ☐ No ☐	Streaming eyes	Yes ☐ No ☐	Fatigue	Yes ☐ No ☐
Itchy eyes	Yes ☐ No ☐	Blocked nose	Yes ☐ No ☐	Poor sleep	Yes ☐ No ☐

Post nasal drip	Yes ☐ No ☐	Coughing	Yes ☐ No ☐	Wheezing	Yes ☐ No ☐
In which months of the year do you <u>currently</u> have symptoms of allergies? (You tick more than one)	☐ Jan ☐ Feb ☐ March ☐ April ☐ May ☐ June ☐ July ☐ Aug ☐ Sept ☐ Oct ☐ Nov ☐ Dec				
(7f) Please tick which <u>type of allergy</u> did you suffer from in the past (NOT currently)					
Nose (hay fever)	Yes ☐ No ☐	Sinusitis	Yes ☐ No ☐	Asthma (allergic)	Yes ☐ No ☐
Skin allergies	Yes ☐ No ☐	Eye allergies	Yes ☐ No ☐	Allergy to plant material	Yes ☐ No ☐
Allergy to foods	Yes ☐ No ☐	Allergy to animals	Yes ☐ No ☐	Allergy to medication	Yes ☐ No ☐

8. Asthma history If you answered **YES** to **question 8** in section E, please complete the following questions (8a. to 8k.) related to your current history of asthma	
(9a) Do you currently suffer from asthma?	Yes ☐ No ☐
(8b) How many years have you suffered from asthma?	(years)
(8c) How was your asthma diagnosed?	☐ A doctor taking a history and performing an examination ☐ Lung function test (blow test) but no exercise ☐ Lung function test (blow test) before and after exercise ☐ Metacholine challenge test ☐ Eucapnic hyperventilation test (rebreathing test) ☐ Other test (Specify: _____)
(8d) Which **type of asthma** do you currently suffer from?	☐ Asthma that occurs at any time but not during exercise ☐ Asthma that occurs at any time including during exercise ☐ Asthma that only occurs during exercise
(8e) Please indicate **how frequently do you currently experience the symptoms** of asthma (shortness of breath, wheezing, coughing or coughing after exercise)?	**Daytime symptoms (per week)** ☐ < 2 / week ☐ 2-4 / week ☐ >4 / week ☐ All the time **Night time symptoms (per month)** ☐ < 1 / month ☐ 2-3 / month ☐ ≥4 / month ☐ All the time **Exercise related symptoms (per 10 exercise sessions)** ☐ <1 per 10 sessions ☐ 2-3 per 10 sessions ☐ ≥4 per 10 sessions
(8f) Please indicate if you had symptoms of asthma that were severe enough to necessitate **hospital admission in the last 12 months**	☐ No hospital admission for asthma in the last 12 months ☐ 1-2 hospital admissions for asthma in the last 12 months ☐ 3-4 hospital admissions for asthma in the last 12 months ☐ >4 hospital admissions for asthma in the last 12 months
(8g) Which **symptoms of asthma** do you currently suffer from?	☐ Wheezing ☐ Dry cough ☐ Shortness of breath ☐ Tight chest ☐ Chest pain ☐ Other (Specify: _____)

(8h) What **medication do you currently use** for your asthma? (you may tick more than one option)	☐ Cortisone inhaler (e.g. Beclate, Becloforte, Becodisks, Becotide, Budeflam, Flixotide, Inflammide, Pulmicort, Qvar, etc) ☐ Salbutamol (bronchodilator) inhaler (e.g. Ventolin, Venteze, Vomax, Airomir, Asthavent etc.) ☐ Salmeterol (bronchodilator) inhaler (Serevent) ☐ Fenoterol (bronchodilator) inhaler (Berotec) ☐ Terbutaline (bronchodilator) inhaler (Bricanyl) ☐ Formoterol (bronchodilator) inhaler (e.g. Foradil, Foratec, Oxis) ☐ Ipratropium (bronchodilator) inhaler (Atrovent) ☐ Tiotropium (bronchodilator) inhaler (Spiriva) ☐ Combined cortisone and bronchodilator inhaler (e.g. Atrovent, Berodual, Combivent, Duolin, Duovent, Seretide, Symbicord) ☐ Cortisone tablets ☐ Bronchodilator tablets ☐ Leukotriene receptor antagonist tablets (e.g. Acccolate, Singulair) ☐ Other inhaler ☐ Other medication (Specify: _____)
(8i) **When do you use your medication** for your asthma?	☐ Daily (irrespective of exercise) ☐ Only before exercise ☐ Other (Specify: _____)
(8j) **How long before an exercise session** do you use your medication for asthma?	min
(8k) Have you obtained **TUE (therapeutic use exemption forms)** for your asthma medication?	Yes ☐ No ☐

9. History of previous collapse If you answered **YES** to **question 9** in section E, please complete the following questions (9a. to 9d.) related to your current history of asthma	
(9a) Have you ever collapsed during training or racing?	☐ Training ☐ Racing ☐ Training and racing
(9b) How many times have you collapsed in training session or races during the last **five years**?	_____ training session _____ races
(9c) How many times have you collapsed in training session or races during the last **12 months** (1 year)?	
(9d) When you collapse, does it mostly occur before of after the finish line / completion of the training session?	☐ Before the finish ☐ After the finish
(9e) What is the cause of you collapse?	☐ Dehydration ☐ Heat illness ☐ Hyponatremia ☐ Low blood pressure ☐ Low blood sugar ☐ Other condition (Specify: _____)

10. History of any current injury that you suffer from

If you answered **YES** to **question 11** in section E, please complete the following questions (10a. to 10g.) related to each of your current injury/ies (Space is provided for two injuries)

Injury 1	
(10a) What was the approximate date when you first became aware of the injury?	Month Year
(10b) Please indicate which side of your body is injured (if applicable)	☐ Right ☐ Left
(10c) Please indicate which anatomical area is currently injured	☐ Head ☐ Elbow ☐ Hamstring ☐ Neck ☐ Forearm ☐ Quadriceps ☐ Face ☐ Wrist ☐ Knee ☐ Front chest ☐ Finger ☐ Shin ☐ Back chest ☐ Lower back ☐ Achilles ☐ Shoulder ☐ Hip ☐ Ankle ☐ Upper arm ☐ Thigh ☐ Foot Other (Specify: _____)
(10d) Please indicate the type of structure that was injured	☐ Muscle ☐ Ligament ☐ Tendon ☐ Joint ☐ Bone Other (Specify: _____)
(10e) Please indicate in which sport (discipline) the injury occurred	☐ Running ☐ Cycling ☐ Swimming Other (Specify: _____)
(10f) Please indicate the severity of the injury (tick one box please)	☐ I only experience symptoms after exercise - Grade 1 ☐ I experience symptoms during exercise, but it does not interfere with exercise - Grade 2 ☐ I experience symptoms during exercise that may interfere with my training/ competition - Grade 3 ☐ I am so painful that I may not be able to train or compete - Grade 4

(10g) Please indicate how your injury was treated to date (you can tick more than one)?	☐ Rest ☐ Tablets ☐ Stretches ☐ Cortisone injection ☐ Physiotherapy ☐ Other injection ☐ Surgery ☐ Orthotics ☐ Strengthening exercises ☐ Equipment change Other (Specify: _____)

Injury 2	
(10a) What was the approximate date when you first became aware of the injury?	Month Year
(10b) Please indicate which side of your body is injured (if applicable)	☐ Right ☐ Left
(10c) Please indicate which anatomical area is currently injured	☐ Head ☐ Elbow ☐ Hamstring ☐ Neck ☐ Forearm ☐ Quadriceps ☐ Face ☐ Wrist ☐ Knee ☐ Front chest ☐ Finger ☐ Shin ☐ Back chest ☐ Lower back ☐ Achilles ☐ Shoulder ☐ Hip ☐ Ankle ☐ Upper arm ☐ Thigh ☐ Foot Other (Specify: _____)
(10d) Please indicate the type of structure that was injured	☐ Muscle ☐ Ligament ☐ Tendon ☐ Joint ☐ Bone Other (Specify: _____)
(10e) Please indicate in which sport (discipline) the injury occurred	☐ Running ☐ Cycling ☐ Swimming Other (Specify: _____)
(10f) Please indicate the severity of the injury (tick one box please)	☐ I only experience symptoms after exercise - Grade 1 ☐ I experience symptoms during exercise, but it does not interfere with exercise - Grade 2 ☐ I experience symptoms during exercise that may interfere with my training/ competition - Grade 3 ☐ I am so painful that I may not be able to train or compete - Grade 4
(11g) Please indicate how your injury was treated to date (you can tick more than one)?	☐ Rest ☐ Tablets ☐ Stretches ☐ Cortisone injection ☐ Physiotherapy ☐ Other injection ☐ Surgery ☐ Orthotics ☐ Strengthening exercises ☐ Equipment change Other (Specify: _____)

Appendix D2 – Medical questionnaire for laboratory testing

GENETIC BASIS OF RANGE OF MOTION MEASUREMENTS

QUESTIONAIRES

PERSONAL DETAILS			
Surname			
First Name			
Postal Address		Postal/ Zip Code	
E-mail address		Phone (day time)	code number
Alternate E-mail address			
Date of birth	yyyy-mm-dd	Cell (Mobile)	
Height	cm	Gender	Male ☐ Female ☐
Weight	kg	Age	yrs
Ethnic group (Only Required and Used for Research Purposes)	Black/African ☐ White ☐ Indian ☐ Mixed Ancestry (Coloured) ☐ Asian ☐ Other ☐		
Ancestry: Tribal or national background (eg Xhosa, Dutch, Zulu, German, Italian)	Father:		Unknown ☐
	Mother:		Unknown ☐
Country of Birth			
Dominant Hand	Left ☐ Right ☐ Both ☐	Dominant Leg	Left ☐ Right ☐ Both ☐
Occupation			

What **percentage** of your **working** day is spent in the following activities?	Sitting: _____ % Standing: _____ % Walking (Lower body activity) _____ % Manual Labour (upper and body activity) _____ %

(If you participate or have participated in more than 6 sports, please complete additional Sporting Details Questionnaires)

SPORTING DETAILS Please record your sporting activities in order of importance			
Type of sport(s) you have participated in (please name)	**Main Sport 1**	**Other Sport 2**	**Other Sport 3**
Current or past participation	Current ☐ Past ☐	Current ☐ Past ☐	Current ☐ Past ☐
Year started participation			
Number of years involved in the sport			
Average hours of training per week			
Position played (if appropriate)			
Playing level (if appropriate)			

Type of sport(s) you have participated in (please name)	**Other Sport 4**	**Other Sport 5**	**Other Sport 6**
Current or past participation	Current ☐ Past ☐	Current ☐ Past ☐	Current ☐ Past ☐
Year started participation			
Number of years involved in the sport			
Average hours of training per week			
Position played (if appropriate)			

Playing level (if appropriate)			

FLEXIBILITY TRAINING HISTORY	
Do you perform flexibility training (regular stretching exercises)?	Yes ☐ No ☐
If YES, please complete the rest of the flexibility training history section below:- If NO, continue completing the questionnaire from the top of page 4.	
On average, how many days a week do you perform a stretching session?	days/week
On average, how many times a day do you perform a stretching session?	times/day
Please tick which muscle groups do you include in your stretching session?	☐ Hamstrings ☐ Quadriceps ☐ Calf (gastrocnemius) ☐ Calf (soleus) ☐ Groin (inner thigh) ☐ Upper body limbs ☐ Other: _____
Please tick when you stretch? (Before, during and/or after exercising. You can tick more than one box)	☐ Before Exercise ☐ During Exercise ☐ After Exercise
When you stretch an individual muscle group, on average, **how long do you hold the stretch** for?	seconds
When you stretch an individual muscle group, on average, **how many times do you stretch the muscle for**?	☐ Once ☐ Twice ☐ 3 times ☐ 4 times ☐ 5 times ☐ 6 or more times

PERSONAL GENERAL MEDICAL HISTORY	
15. Do you currently suffer from high blood pressure?	Yes ☐ No ☐
16. Do you currently suffer from angina/heart attack?	Yes ☐ No ☐
17. Do you currently suffer from asthma?	Yes ☐ No ☐
18. Do you currently suffer from emphysema?	Yes ☐ No ☐
19. Do you currently suffer from rheumatoid arthritis?	Yes ☐ No ☐
20. Do you currently suffer from osteoarthritis (wear and tear)?	Yes ☐ No ☐
21. Do you currently suffer from malignant disease (cancer)?	Yes ☐ No ☐
22. Do you currently suffer from elevated blood cholesterol?	Yes ☐ No ☐
23. Do you currently suffer from adrenal disorders?	Yes ☐ No ☐
24. Do you currently suffer from diabetes mellitus?	Yes ☐ No ☐
25. Do you currently suffer from thyroid disorders?	Yes ☐ No ☐
26. Do you currently suffer from renal disease?	Yes ☐ No ☐
27. Do you currently suffer from amyloidosis?	Yes ☐ No ☐
28. Please tick in which anatomical area you had **surgery** performed, if ever.	☐ Gastric (stomach) ☐ Oesophageal (swallowing pipe) ☐ Small bowel ☐ Large bowel (colon) ☐ Rectum ☐ Gallbladder ☐ Pancreas ☐ Liver ☐ Abdomen (general) ☐ Wrist ☐ Head ☐ Finger ☐ Neck ☐ Lower back ☐ Face ☐ Hip ☐ Front chest ☐ Thigh ☐ Back chest ☐ Knee ☐ Shoulder ☐ Lower leg ☐ Upper arm ☐ Achilles ☐ Elbow ☐ Ankle ☐ Forearm ☐ Foot ☐ Other (Specify: _____)

History of medication and supplement use			
What medication, if any, are you currently using? (please list)	Name of medication		Years taken
Have you ever used oral corticosteroids (cortisone tablets)? (If **yes**, how long ago?)	Yes ☐ No ☐	☐ 3 months ☐ 6 months ☐ 12 months ☐ 24 or more months	
Have you ever been given an injection with corticosteroids? (If **yes**, how long ago?)	Yes ☐ No ☐	☐ 3 months ☐ 6 months ☐ 12 months ☐ 24 or more months	
Have you ever been given an injection of corticosteroids in or around the **Achilles** tendon? (If **yes**, how many times?)	Yes ☐ No ☐	☐ Once ☐ Twice ☐ 3 times ☐ >3 times	
Have you ever used fluoroquinoline antibiotics? (refer to the following list)	Yes ☐ No ☐	☐ 3 months ☐ 6 months ☐ 12 months ☐ 24 or more months	
What allergies do you have? (please list)			

List of some fluoroquinoline antibiotics:		
ADCO-CIPRIN	CIPROBAY	SANDOZ CIPROFLOXACIN
AVELON	CIPROGEN	TAFLOC
BACTIDRON	CPL ALLIANCE CIPROFLOXACIN	TARIVID
CIFLOC	DYNAFLOC	TAVANIC
CIFRAN	FACTIVE	TEQUIN
CIPLA-CIPROFLOXACIN	FLOXIN	UNIQUIN
CIPLOXX	MAXAQUIN	UTIN-400
CIPRO-HEXAL	NOROXIN	ZANOCIN
	ORPIC	

LIFESTYLE AND HABITS HISTORY

Please indicate your smoking status	Current smoker ☐	Ex smoker ☐	Never smoked ☐
If you answered yes, (past or current smoker) please complete the section on the right	Number of years of smoking:	If stopped, how many years ago:	
	What is (was) the average number of cigarettes per day:		

FAMILY MEDICAL HISTORY		
Have any of your blood (biological) relatives <u>ever</u> had the following? **Please tick yes or no. If yes, please tick the relationship of that person to you (You may tick more than one of the relationship blocks).**		
Description		**If Yes, please indicate the relationship**
Chronic Achilles tendon injury	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Achilles tendon rupture	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Any ligament injury	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Arthritis	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Elevated blood cholesterol	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother

TENDON AND LIGAMENT INJURY HISTORY						
Please tick which **tendon/s** you have injured? (next column on the tright) Also indicate (tick) if your injured tendon was longsatnding pain (tendinopathy) or an acute tear/rupture		**Tendon**	**L**	**R**	**Longstanding Pain (Tendinopathy)**	**Acute Tear/ Rupture**
	Foot and ankle:	☐ Achilles tendon	☐	☐	☐	☐
		☐ Tibialis posterior	☐	☐	☐	☐
		☐ Plantar fascia	☐	☐	☐	☐
	Knee:	☐ Patellar tendon	☐	☐	☐	☐
	Elbow and wrist:	☐ Wrist extensor tendon	☐	☐	☐	☐
	Shoulder:	☐ Subscapularis	☐	☐	☐	☐
		☐ Suprasinatus	☐	☐	☐	☐
		☐ Infraspinatus	☐	☐	☐	☐
		☐ Teres minor	☐	☐	☐	☐
	Other: _____		☐	☐	☐	☐
Please tick which	**Ligament**		**L**	**R**	**Sprain**	**Complete Tear**

ligament/s you have injured? (next column on the tright) Also indicate if your sprained or completely tore the ligamant.	☐ Shoulder ligaments ☐ Elbow ligaments ☐ Wrist ligaments ☐ Finger ligaments ☐ Knee (ACL) ☐ Knee (MCL) ☐ Knee (PCL) ☐ Knee (LCL) ☐ Ankle lateral ligaments ☐ Ankle medial ligaments ☐ Spinal ligaments ☐ Other: _____
Please tick if you have you ever suffered from any of the following **joint capsule** injuries?	☐ Acute shoulder dislocation ☐ Chronic shoulder instability ☐ Chronic ankle instability ☐ Other: _____
Do you suffer from any other **connective tissue or rheumatological diseases** or disorders? (If yes, please specify which one)	Yes ☐ No ☐ (refer to the list on the next page) (If yes, specify: _____)

List of some Connective Tissue and/or Rheumatic Diseases and Disorders

Ankylosing Spondylitis	Lipid Storage Diseases	Pseudogout
Aspartylglycosaminuria (AGU)	Marfan Syndrome	Reactive Arthritis
Behcet's Syndrome	Menkes Kinky Hair Syndrome	Reiter's Syndrome
Crohn's Disease	Mucopolysaccharidoses	Relapsing Polychrondritis
Discoid Lupus Erythematosus	Myopathies and Dystrophies	Scleroderma
Ehlers-Danlos syndrome (EDS)	Ochronosis (Homocystinuria)	Sjogren's Syndrome
Eosinophilic Fascitis	Osteogenesis imperfecta (OI)	Systemic Lupus Erythematosus (SLE)
Giant Cell (Temporal) Arthritis	Polyarteritis Nodosa	Systemic Sclerosis
Gout	Polymyalgia Rheumatica	Wegener's Granulomatosis
Hypersentive Vasulatis	Polymyositis & Dermatomyositis	

CURRENT INJURY

Current Injury 1	
What was the approximate date when you first became aware of the injury?	Month Year
Please indicate which side of your body is injured (if applicable)	☐ Right ☐ Left
Please indicate which anatomical area is currently injured	☐ Head ☐ Elbow ☐ Hamstring ☐ Neck ☐ Forearm ☐ Quadriceps ☐ Face ☐ Wrist ☐ Knee ☐ Front chest ☐ Finger ☐ Shin ☐ Back chest ☐ Lower back ☐ Achilles ☐ Shoulder ☐ Hip ☐ Ankle ☐ Upper arm ☐ Thigh ☐ Foot Other (Specify: _____)
Please indicate the type of structure that was injured	☐ Muscle ☐ Ligament ☐ Tendon ☐ Joint ☐ Bone Other (Specify: _____)
Please indicate in which sport (discipline) the injury occurred	☐ Running ☐ Cycling ☐ Swimming Other (Specify: _____)
Please indicate the severity of the injury (tick one box please)	☐ I only experience symptoms after exercise - Grade 1 ☐ I experience symptoms during exercise, but it does not interfere with exercise - Grade 2 ☐ I experience symptoms during exercise that may interfere with my training/ competition - Grade 3 ☐ I am so painful that I may not be able to train or compete - Grade 4
Please indicate how your injury was treated to date (you can tick more than one)?	☐ Rest ☐ Tablets ☐ Stretches ☐ Cortisone injection ☐ Physiotherapy ☐ Other injection ☐ Surgery ☐ Orthotics ☐ Strengthening exercises ☐ Equipment change Other (Specify: _____)

Current Injury 2	
What was the approximate date when you first became aware of the injury?	Month Year
Please indicate which side of your body is injured (if applicable)	☐ Right ☐ Left
Please indicate which anatomical area is currently injured	☐ Head ☐ Elbow ☐ Hamstring ☐ Neck ☐ Forearm ☐ Quadriceps ☐ Face ☐ Wrist ☐ Knee ☐ Front chest ☐ Finger ☐ Shin ☐ Back chest ☐ Lower back ☐ Achilles ☐ Shoulder ☐ Hip ☐ Ankle ☐ Upper arm ☐ Thigh ☐ Foot Other (Specify: _____)
Please indicate the type of structure that was injured	☐ Muscle ☐ Ligament ☐ Tendon ☐ Joint ☐ Bone Other (Specify: _____)
Please indicate in which sport (discipline) the injury occurred	☐ Running ☐ Cycling ☐ Swimming Other (Specify: _____)
Please indicate the severity of the injury (tick one box please)	☐ I only experience symptoms after exercise - Grade 1 ☐ I experience symptoms during exercise, but it does not interfere with exercise - Grade 2 ☐ I experience symptoms during exercise that may interfere with my training/ competition - Grade 3 ☐ I am so painful that I may not be able to train or compete - Grade 4
Please indicate how your injury was treated to date (you can tick more than one)?	☐ Rest ☐ Tablets ☐ Stretches ☐ Cortisone injection ☐ Physiotherapy ☐ Other injection ☐ Surgery ☐ Orthotics ☐ Strengthening exercises ☐ Equipment change Other (Specify: _____)

Appendix E – Data sheet for testing

Blood sample no.:			Time:
Contact number:			Date:
Recent injuries?	Where?	When?	Height:
Exercised past 24 hrs?	M/Mod/Int UB/LB	Typical/Atypical	Weight:
Stretched past 24 hrs?	UB/LB		Waist circumference:
Analgesic 24 hours?			Temperature
Transport to venue?			Age:

Clinical criteria for BJHS	Yes	No
1. Can you now (or could you ever) place your hands flat on the floor without bending your knees?		
2. Can you now (or could you ever) bend your thumb to touch your forearm?		
3. As a child did you amuse your friends by contorting your body into strange shapes OR could you do the splits?		
4. As a child or teenager did your shoulder or kneecap dislocate on more than one occasion?		
5. Do you consider yourself double jointed?		

Are you currenlty involved in an activity on a regular basis that involves a throwing action? _____

Throwing Hand	Right	Left
Kicking leg	Right	Left

Arm intervention	Right	Left
Leg intervention	Right	Left

Sit and Reach

1	**2**

Straight Leg Raise

I	**C**	**P-I**	**P-C**

Shoulder Rotation

Control		**Intervention**		**Post-intervention**		**Post-control**	
I.R	**E.R**	**I.R**	**E.R**	**I.R**	**E.R**	**I.R**	**E.R**

Appendix F – Extra tables and figures for Chapter 2

Appendix F1 - Bland-Altman analysis for SR test

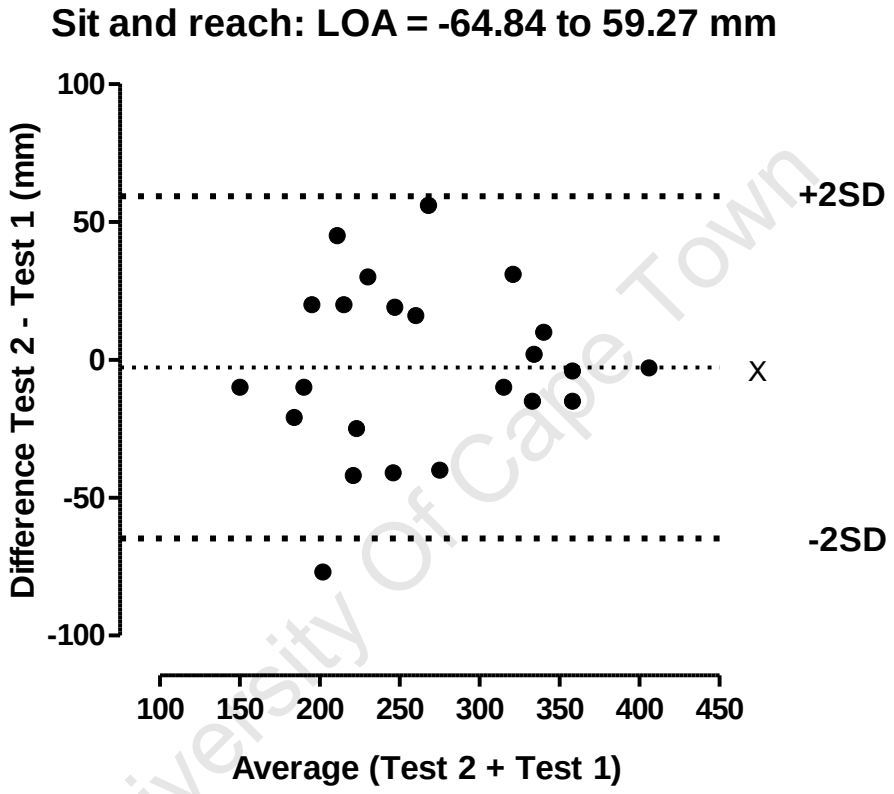

Figure F.1. Bland-Altman analysis for SR ROM test-retest reliability assessment.

Appendix F2 - Reported injuries

Table F.1: Reported incidence of common injuries by means of self-reported questionnaire in entire sample

Injury by anatomical site [a]	N	%
Knee	20	25.6
Lower back	8	10.3
Achilles	7	9.0
Hamstring/Thigh	7	9.0
Shoulder	5	6.4
Hip	5	6.4
Calf	5	6.4
Ankle	4	5.1
Finger/wrist	4	5.1
Elbow	3	3.8
Foot	3	3.8
Shin	2	2.6
Groin	1	1.3
ITB [b]	1	1.3
Rhomboid Area	1	1.3
Siatica [b]	1	1.3
Back	1	1.3

[a] Some subjects listed more than one anatomical site.
N=number
[b] While it is acknowledged that these are not recognized anatomical sites, they were actual answers that were listed in the "other" section of this question.

Table F.2: Reported incidence of injury, by anatomical structure for entire sample.

Injury by structure [a]	N	%
Muscle	28	36.8
Ligament	17	22.4
Tendon	14	18.4
Bone	8	10.5
Joint	4	5.3
Impingement [b]	1	1.3
Cartilage	1	1.3
Lower back cushion [b]	1	1.3
Bursitis [b]	1	1.3
Dislocation [b]	1	1.3

[a] Some subjects listed more than one structure.
N=number
[b] While it is acknowledged that these are not recognized anatomical structures, they were actual answers that were listed in the "other" section of this question.

Appendix F3 - Reported sport participation

Table F.3: List of all sports reported by cohort and their associated lower body ROM. If a reference is not available, the sport is compared to a sport with similar demands, and referenced as such.

Decrease ROM	Reference	Increased ROM	Reference	Unknown
Action netball	Soccer [13]	Ballet	Ballet [52]	Aerobics
Basketball	Review [64]	Dancing	Ballet [52]	Ballroom dancing
Cricket	Soccer [13]	Judo	Aikido [123]	Canoe/Paddling
Cycling	Cycling [2]	Jujitsu	Aikido [123]	Climbing
Hiking	Running [27]	Muay Thai	Aikido [123]	Golf
Hockey	Soccer [13]	Pilates	Ballet [52]	Gym
Netball	Soccer [13]	Rock Climbing	Aikido [123]	Horse Riding
Powerlifting	Elite orienteers [85]	Swimming	Swimming [53]	Ice skating
Rugby	Soccer [13]			Kata Boxing
Running	Running [27]			Life Saving
Soccer	Soccer [13]			Rowing
Squash	Soccer [13]			Stepping
Touch Rugby	Soccer [13]			Surfing
Track and field	Running [27]			Tennis
Triathlon	Running [27]			Walking

Appendix F4 - SLR sub-sample

Table F.4. Comparison of variables that were significantly correlated with ROM measures in SLR sub-sample and the rest of the sample.

	SLR sub-sample	Remainder of sample (excl. SLR)	p-value [a]
Gender (% male)	55.7 (34)	64.5 (169)	0.202
Exercised in past 24 hours (% Yes)	17.2 (10)	37.8 (91)	**0.028**
Effect of sport on lower body ROM			
Decreasing	82.9 (29)	93.2 (150)	0.683
Increasing	17.1 (6)	6.0 (11)	0.823

[a] SLR sub-sample vs remainder or cohort

Appendix F5 - Correlations between intrinsic and extrinsic factors and ROM measurements

Table F.5: Correlations between intrinsic/extrinsic factors and ROM assessments in males.

	Dom. SLR	Dom. ShIR –	Dom. ShER-	Non-dom. ShIR	Non-dom. ShER –
Age	r=-0.35 N=32 p=0.053	r=0.02 N=102 p=0.847	r=-0.11 N=102 p=0.289	r=-0.01 N=102 p=0.945	r=-0.17 N=102 p=0.081
Height	r=0.02 N=33 p=0.935	r=0.06 N=102 p=0.554	r=-0.02 N=102 p=0.852	r=-0.06 N=102 p=0.537	**r=-0.20** **N=102** **p=0.047**
Weight	r=0.05 N=33 p=0.790	r=-0.02 N=102 p=0.833	r=-0.13 N=102 p=0.193	r=-0.10 N=102 p=0.332	r=-0.15 N=102 p=0.133
BMI	r=0.03 N=31 p=0.863	r=-0.04 N=100 p=0.696	r=-0.15 N=100 p=0.137	r=-0.09 N=100 p=0.401	r=-0.08 N=100 p=0.450
Waist	r=0.04 N=17 p=0.867	r=-0.13 N=86 p=0.243	r=-0.08 N=86 p=0.488	r=-0.06 N=86 p=0.601	r=-0.15 N=86 p=0.178
Flex. training	r=0.27 N=30 p=0.154	r=0.17 N=92 p=0.097	r=0.05 N=92 p=0.669	r=-0.05 N=92 p=0.613	r=0.05 N=92 p=0.648

Bold font emphasizes data are significantly correlated at $p<0.05$.

I.R. – internal rotation; E.R. – external rotation; T.R. – Total Rotation, dom. – dominant; SR – Sit and Reach; SLR – Straight Leg Raise; BMI – Body Mass Index; min – minutes; ROM – range of motion; Flex. – flexibility.

Table F.6: Correlations between intrinsic/extrinsic factors and ROM assessments in females.

	SLR - Dom	IR – dom. shoulder	ER- dom. shoulder	IR – non-dom. shoulder	ER – non-dom. shoulder
Age	r=-0.14 N=26 p=0.490	r=-0.19 N=73 p=0.115	r=-0.08 N=75 p=0.498	**r=-0.23** **N=75** **p=0.049**	r=0.04 N=75 p=0.706
Height	r=-0.18 N=26 p=0.368	r=-0.01 N=72 p=0.946	r=-0.00 N=74 p=0.970	r=-0.17 N=74 p=0.140	r=-0.12 N=74 p=0.324
Weight	r=-0.22 N=26 p=0.277	r=0.07 N=72 p=0.586	r=0.14 N=74 p=0.250	r=0.03 N=74 p=0.833	r=0.05 N=74 p=0.667
BMI	r=-0.13 N=26 p=0.518	r=0.09 N=72 p=0.464	r=0.16 N=74 p=0.163	r=0.14 N=74 p=0.251	r=0.13 N=74 p=0.260
Waist	r=-0.30 N=20 p=0.202	r=0.17 N=66 p=0.176	r=0.04 N=68 p=0.772	r=0.14 N=68 p=0.252	r=0.12 N=68 p=0.333
Flex. training	r=-0.22 N=22 p=0.337	r=0.09 N=65 p=0.484	r=-0.23 N=67 p=0.063	r=-0.21 N=67 p=0.089	r=0.07 N=67 p=0.573

Bold font emphasizes data are significantly correlated at $p<0.05$.

I.R. – internal rotation; E.R. – external rotation; T.R. – Total Rotation, dom. – dominant; SR – Sit and Reach; SLR – Straight Leg Raise; BMI – Body Mass Index; min – minutes; ROM – range of motion; Flex. – flexibility.

Appendix F6

Table F.7: Correlations between ROM assessments.

	Dom. SLR	Non-dom. - SLR	Dom. Sh IR	Dom. Sh ER	Non-dom. Sh IR	Non-dom. ShER
SR	**r=0.81** **N=53** **p<0.001**	**r=0.74** **N=53** **p<0.001**	r=0.09 N=170 p=0.231	**r=0.26** **N=172** **p=0.001**	r=0.13 N=172 p=0.095	**r=0.34** **N=172** **p=0.000**
Dom. SLR		**r=0.78** **N=61** **p<0.001**	**r=0.32** **N=60** **p=0.014**	**r=0.51** **N=60** **p<0.001**	r=0.19 N=60 p=0.140	**r=0.64** **N=60** **p<0.001**
Non-dom. - SLR	**r=0.78** **N=61** **p<0.001**		r=0.40 N=60 p=0.002	**r=0.45** **N=60** **p<0.001**	r=0.19 N=60 p=0.142	**r=0.66** **N=60** **p<0.001**
Dom. ShIR	**r=0.32** **N=60** **p=0.014**	**r=0.40** **N=60** **p=0.002**		**r=0.29** **N=177** **p<0.001**	**r=0.55** **N=177** **p<0.001**	**r=0.47** **N=177** **p<0.001**
Dom. ShER	**r=0.51** **N=60** **p<0.001**	**r=0.45** **N=60** **p<0.001**	**r=0.29** **N=177** **p<0.001**		**r=0.40** **N=179** **p<0.001**	**r=0.68** **N=179** **p<0.001**
Non-dom. Sh IR	r=0.19 N=60 p=0.140	r=0.19 N=60 p=0.142	**r=0.55** **N=177** **p<0.001**	**r=0.40** **N=179** **p<0.001**		**r=0.36** **N=179** **p<0.001**

Bold font emphasizes data are significantly correlated at p<0.05.

I.R. - internal rotation; E.R. - external rotation; T.R. - Total Rotation, dom. - dominant; SR - Sit and Reach; SLR - Straight Leg Raise; BMI - Body Mass Index; min - minutes; ROM - range of motion; Flex. - flexibility.

Appendix G - DNA Extraction from Whole Blood

Last modified March 11 2005

Reagents

1. TKM1 Buffer (pH 7.6)

	Final Conc.	**MW**	**For 500ml**	**For 1000ml**
Tris-HCl	10mM	121.00	0.6056	1.2112
KCl	10mM	74.56	0.3728	0.7456
$MgCl_2.6H_2O$	10mM	203.20	1.016	2.032
EDTA	2mM	372.24	0.372	0.744
dH_2O			to 500ml	To 1000ml

- Autoclave
- Make up 1 volume which includes 2.5% NP40 and 1 volume without NP40

2. TKM2 Buffer (pH 7.6)

	Final Conc.	**MW**	**For 200ml**
Tris-HCl	10mM	121.00	0.242
KCl	10mM	74.56	0.149
$MgCl_2.6H_2O$	10mM	203.20	0.406
EDTA	2mM	372.24	0.1488
NaCl		58.44	4.675
dH_2O			to 200 ml

- Autoclave

3. 10% SDS

	Final Conc.	**MW**	**For 200ml**
SDS	10%		20
dH_2O			to 200 ml

- Autoclave

4.1X TE buffer (pH 8.0)

	Final Conc.	**MW**	**For 100ml**
Tris-HCl	10mM	121.00	0.121
EDTA	1mM	372.24	0.037
dH_2O			to 100 ml

- Autoclave

5. 5M $NaClO_4$

	Final Conc.	**MW**	**For 100ml**
$NaClO_4$	5M	122.4	61.2
dH_2O			to 100 ml

- Autoclave

5. Other Chemicals and Reagents

- Chloroform (molecular grade)
- NP40
- Absolute ethanol

Protocol

1. Draw 5mls of blood into an EDTA vacutainer tube (Purple top).
2. Blood can be stored at 4ºC up to 1 week before the DNA is extracted.
3. Transfer the blood to a sterile 15ml polypropolene tube.
4. Add 2 volumes (10ml) of TKM1 buffer containing 2.5% NP40.
5. Mix by inverting several times and incubate at room temperature for 10 minutes in order to enhance the haemolysis of red blood cells.
6. Centrifuge at 3000rpm (1200Xg) at room temperature for 10 minutes.
7. Decant off the supernatant containing leaving the white pellet at the bottom of the tube.
8. Add 1 volume (5ml) of TKM1 buffer (without NP40).
9. Invert and vortex the solution.
10. Centrifuge at 3000rpm (1200Xg) at room temperature for 10 minutes.
11. Decant the supernatant leaving the white pellet in the bottom of the tube.
12. Repeat steps 7-10 until the pellet in the bottom of the tube is clean and white.
13. Add 800ul of TKM2 buffer and 50ul of the 10% SDS solution.
14. Vortex and then **mix using a blue pipette tip** in order to assist in the lyses of the white blood cells.
15. Incubate for 60 minutes at 55ºC in a water bath. Make sure the pellet is totally dissolved before moving on.
16. Add 150ul of 5M $NaClO_4$.
17. Add 500ul of molecular biology grade chloroform.
18. Vortex the solution.
19. Transfer the solution to sterile 1.5ml microfuge tubes.
20. Centrifuge at 1300rpm at room temperature for 5 minutes.
21. Carefully transfer 500ul of the top aqueous phase to a new sterile microfuge tube.
22. Add 1ml of absolute ethanol.
23. Invert until DNA precipitates.
24. Centrifuge at 1300rpm at room temperature for 5-10 minutes.
25. Carefully tip off supernatant leaving the pellet in the bottom of the tube.
26. Allow pellet to air dry completely.
27. Add 200ul of 1XTE buffer.

28. Incubate the tubes at 65ºC for 15 minutes in a heating block.
29. Store DNA at 4ºC.

Reference: Lahiri et al.(1991) Nucleic acids research 19:54444

Appendix H - extra tables and figures for Chapter 3

Appendix H1 – Hardy-Weinberg test for population stratification: *Bst*UI RFLP

Results from GENEPOP

Tue Feb 16 22:17:25 WST 2010

```
Genepop  version 4.0.10: Hardy-Weinberg test

File: 221726 (BSTUI)

Number of populations detected:    1
Number of loci detected:           1


Estimation of exact P-Values by the Markov chain method.
--------------------------------------------
Markov chain parameters for all tests:
Dememorization:              1000
Batches:                     100
Iterations per batch:        1000
Hardy Weinberg: Probability test
        ************************



=========================================
     Results by population
=========================================


 Pop : c302
-----------------------------------------
                             Fis estimates
                            ---------------
locus       P-val   S.E.    W&C     R&H     Steps
----------- ------- ------- ------- ------- ------
RS          0.5545  0.0070   0.0369  0.0369  90674 switches

Normal ending
```

Appendix H2 – Hardy-Weinberg test for population stratification: *Dpn*II RFLP

Results from GENEPOP

Tue Feb 16 22:34:18 WST 2010

```
Genepop  version 4.0.10: Hardy-Weinberg test

File: 223418 (DPNII)

Number of populations detected:    1
Number of loci detected:           1


Estimation of exact P-Values by the Markov chain method.
--------------------------------------------
Markov chain parameters for all tests:
Dememorization:              1000
Batches:                     100
Iterations per batch:        1000
Hardy Weinberg: Probability test
        ************************



=========================================
     Results by population
=========================================


 Pop : c298
-----------------------------------------
                             Fis estimates
                            ---------------
locus       P-val   S.E.    W&C     R&H     Steps
----------- ------- ------- ------- ------- ------
RS          0.7743  0.0036   0.0188  0.0189  88717 switches

Normal ending
```

Appendix H3

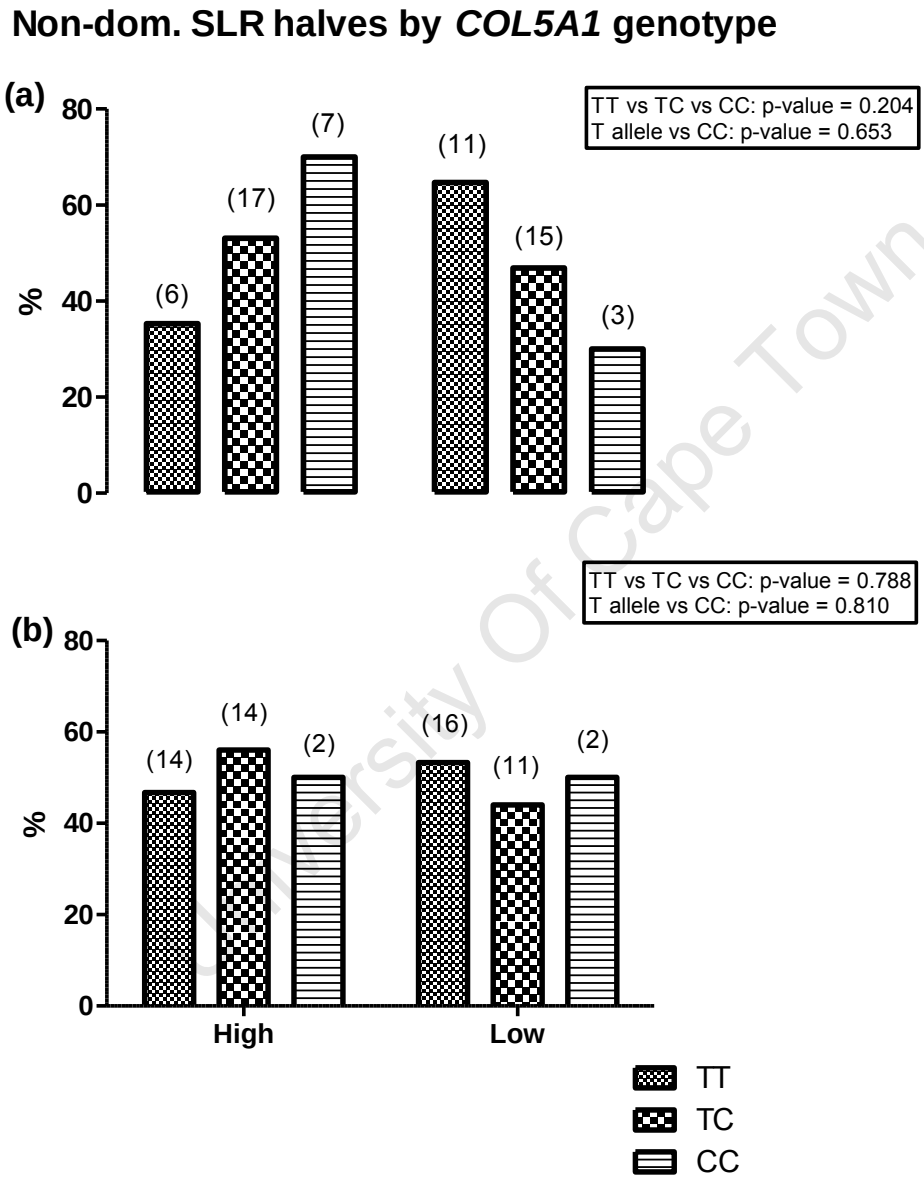

Figure G.1: The *COL5A1* **(a)** *Bst*UI and **(b)** *Dpn*II restriction fragment length polymorphism (RFLP) genotype distributions within the non-dominant straight leg raise (SLR) High and Low halves. The number of samples (N) is indicated in parenthesis above each bar. Cohort is divided into halves due to low sample numbers.

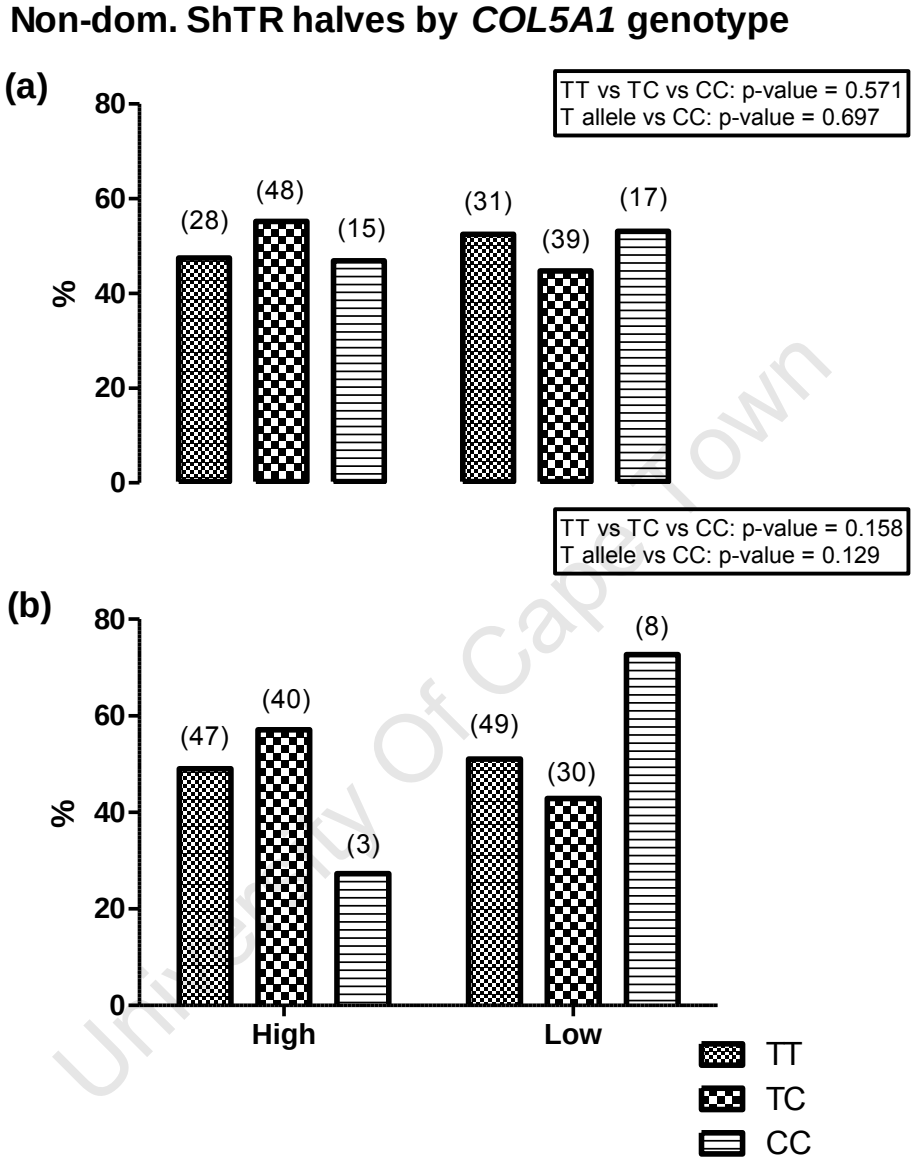

Figure G.2: The *COL5A1* **(a)** *Bst*UI and **(b)** *Dpn*II restriction fragment length polymorphism (RFLP) genotype distributions within the non-dominant shoulder total rotation (ShTR) High and Low halves. The number of samples (N) is indicated in parenthesis above each bar. Cohort is divided into halves due to poor repeatability of this assessment.

Appendix H4

Table H.1. Correlations and interactions between *COL5A1 Bst*UI RFLP genotypes, non-genetic factors for SR ROM

	TT	TC	CC	Interaction p-value [a]
	r=-0.15	r=-0.11	r=0.25	
Age (years)	N=99	N=135	N=59	**0.024**
	p=0.149	p=0.213	p=0.058	
	r=-0.21	**r=-0.25**	r=-0.23	
Height (m)	N=85	**N=105**	N=46	0.826
	p=0.055	**p=0.010**	p=0.117	
	r=-0.18	r=-0.16	r=-0.20	
Weight (kg)	N=85	N=105	N=46	0.544
	p=0.107	p=0.105	p=0.186	
	r=-0.11	r=-0.00	r=-0.07	
BMI (kg/m^2)	N=84	N=104	N=46	0.768
	p=0.334	p=0.994	p=0.632	
	r=0.25	r=-0.04	r=0.10	
Flex training (min/wk)	**N=79**	N=106	N=44	0.114
	p=0.028	p=0.720	p=0.532	
	r=0.10	r=-0.21	r=-0.14	
Two Oceans Ultra-marathon Finish Time	N=32	N=48	N=24	0.424
	p=0.600	p=0.144	p=0.517	

Bold font emphasizes data are significantly correlated at p<0.05.
BMI – Body Mass Index; min - minutes; Flex – flexibility; min – minutes; wk – week; cm – centimeters; mm – millimeters; m - meters
[a] Non-genetic factor vs Genotype (T allele and CC genotype)

Table G.2. Correlations and interactions between *COL5A1 Dpn*II RFLP genotypes, non-genetic factors and SR measurements

	TT	TC	CC	Interaction p-value [a]
	r=-0.07	r=-0.05	r=0.09	
Age (years)	N=148	N=114	N=25	0.351
	p=0.411	p=0.588	p=0.665	
	r=-0.20	**r=-0.26**	r=-0.39	
Height (m)	**N=122**	**N=90**	N=18	0.627
	p=0.025	**p=0.014**	p=0.114	
	r=-0.11	**r=-0.26**	r=-0.19	
Weight (kg)	N=122	**N=90**	N=18	0.783
	p=0.227	**p=0.013**	p=0.447	
	r=0.00	r=-0.17	r=0.06	
BMI (kg/m^2)	N=120	N=90	N=18	0.627
	p=0.989	p=0.105	p=0.805	
	r=0.14	r=0.00	r=0.03	
Flex. training (min/wk)	N=116	N=88	N=17	0.969
	p=0.134	p=0.991	p=0.898	
	r=0.00	r=-0.18	r=-0.03	
Two Oceans Ultra-marathon Finish Time	N=49	N=24	N=12	0.714
	p=0.982	p=0.245	p=0.916	

Bold font emphasizes data are significantly correlated at p<0.05.
BMI - Body Mass Index; min - minutes; Flex – flexibility; min – minutes; wk – week; cm – centimeters; mm – millimeters; m – meters.
[a] Non-genetic factor vs Genotype (T allele and CC genotype)

Appendix H5

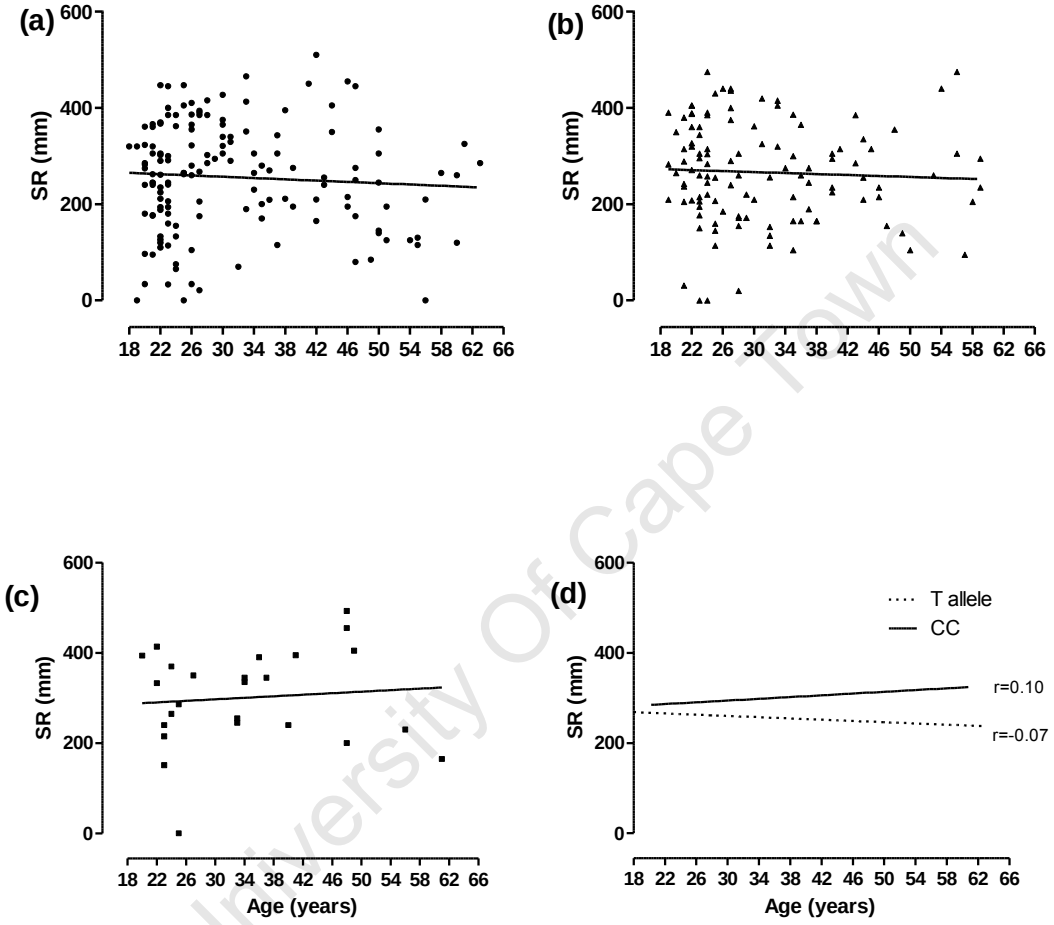

Figure H.3. The relationship between sit and reach measurement with increasing age for each *COL5A1 Dpn*II RFLP genotype. **(a)** TT genotype, **(b)** TC genotype, **(c)** CC genotype and **(d)** T allele (TT and TC) vs CC genotype. r value is the correlation value the two factors (SR and age).

Appendix H6

Table H.3: Correlations of non-genetic intrinsic factors with sit and reach (SR) measurements. Sample is divided by age category into "young" (age<35 years) and "old" (age ≥ 35 years).

	Young	**Old**
Age (years)	r=0.12	r=-0.06
	N=204	N=109
	p=0.098	p=0.520
Height (m)	**r=-0.18**	**r=-0.45**
	N=183	**N=61**
	p=0.015	**p=0.000**
Weight (kg)	r=-0.11	**r=-0.43**
	N=183	**N=61**
	p=0.125	**p=0.001**
BMI (kg/m^2)	r=-0.02	r=-0.25
	N=182	N=60
	p=0.743	p=0.058
Waist circumference (cm)	**r=-0.19**	r=-0.37
	N=137	N=13
	p=0.023	p=0.219
Flex. training (min/wk)	**r=0.16**	r=0.10
	N=166	N=70
	p=0.041	p=0.428

Values are expressed as average ± standard deviation or as a frequency. The number of subjects (N) is in parentheses. Age, height, weight and BJHS score and limb dominance were obtained or measured during the first visit. Body mass index (BMI) was calculated as kilograms per metre squared. Country of birth, occupation, limb dominance and injury data were self-reported in a questionnaire.
Dom. – dominant, Flex. – flexibility, min – minutes wk – week

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